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Original Research Article

FORMULATION AND EVALUATION OF IN-SITU NASAL GEL OF SALBUTAMOL SULPHATE

Shakir Shoukat Sikilkar*, Archana Narayan Barhate, Amruta Chandrakant Menkudale

M.Pharmacy ,Svpm's College Of Pharmacy, Malegaon (Bk), Baramati, Pune, 411315.

ABSTRACT

This study aimed to formulate and evaluate nasal drug delivery system containing Salbutamol Sulphate for improving the bioavailability and 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%. Hence, an *In situ* nasal gel of Salbutamol Sulphate was prepared to enhance its bioavailability as well as reducing the dosing frequency. Natural polymer and its carboxymethyl derivative were used to enhance mucoadhesive and poloxamer 407 was used for its sol gel property. DRG was successfully derivatized to CMDRG by etherification reaction. Drug and polymers were characterized by FTIR and DSC analysis. Salbutamol Sulphate was selected for formulation of in situ gel. DRG and CMDRG were used in varying concentration as a mucoadhesive polymer. The result revealed that as the Increase in concentration of polymers should decrease in gelation temperature of formulations while increase in concentration of polymers produced increase in mucoadhesion force of all formulations. pH of all formulations were in the range of 5.3 and 6.5. The drug content of all formulation was found to be 93.6% - 96.66%. The drug release was extended up to 8 hrs.

Keywords: Salbutamol sulphate, Bioavailability, *In-situ nasal gel,* etherification, *Delonix regia* gum, mucoadhesion.

INTRODUCTION

Therapy through intranasal administration has been an accepted form of treatment in the Ayurvedic system of Indian Medicine. In recent years many drugs have been shown to achieve better systemic bioavailability through nasal route than by oral administration[1].

Conventionally the nasal cavity used for the treatment of lacal disease, such as rhinitis and nasal congestion. However, in the past few decades nasal drug delivery has been paid much more attention as a promising drug administration route for the systemic therapy. This is due to anatomy and physiology of the nasal passage, such as the large surface area, highly vascularized epithelium, porous endothelial membrane and the avoidance of first-pass metabolism [2].

The nasal drug delivery shows many advantages such as nasal drug delivery avoids degradation of drug in gastrointestinal tract resulting from acidic or enzymatic degradation, it avoids degradation of drug resulting from hepatic first-pass metabolism, results in rapid in rapid absorption and onset of action, results in higher bioavailability thus uses lower doses of drug, easily accessible, non-invasive route, self medication is possible through this route, direct transport in to systemic circulation and CNS is possible, it offers lower risk of overdose, it does not have any complex formulation requirement [2, 3, 4].

MATERIALS AND METHOD

Salbutamol Sulphate and Poloxamer 407 were purchased from Yarrow chem Product Mumbai, Delonix Regia seeds were collected from local area of Baramati, Benzalkonium Chloride was obtained from Research Fine Chem Industries Mumbai, Sodium Chloro Acetate, Ethanol and Methanol were purchased from Loba Chemie Pvt Ltd Mumbai.

Gel was prepared by using Magnetic Stirrer REMI 1 MLH, Ex-vivo diffusion study was performed using Nasal diffusion cell, UV Visible spectrophotometer Shimadzu UV-1700, FTIR spectrophotometer Shimadzu 1700, Differential Scanning Calorimetry PerkinElmer 4000 was used.

METHOD

Carboxymethylation of Delonix regia Gum:

The Delonix regia gum was isolated by proposed method [5]. The Delonix regia gum was modified by carboxymethylation using SCA under heterogeneous conditions. A 70 g sample of gum was allowed to swell in 400 ml of 2-propanol, under constant agitation. After initial swelling, 24.8 g of NaOH solution (40%) was added over a period of 20 min, and the mixture was left to stand for 30 min at room temperature to allow further swelling. Sixty grams of SCA solution (40%) was added drop wise over 30 min, and the mixture was allowed to react for 1 h. The reaction temperature was then raised to 70 C over 1h, and the reaction was allowed to proceed for 3 h at 70^0 C. The mixture was cooled to room temperature and filtered. The resulting solid was washed and soaked in 80% (v/v) methanol/water for 30 min to remove inorganic salts. The carboxymethylated gum was recovered by filtration, washed with 99.9% methanol, and dried overnight at 60°C in an oven [6].

Preparation of thermoreversible Salbutamol Sulphate gel:

Salbutamol Sulphate along with mucoadhesive polymer and preservative were stirred in the calculated amount of distilled water at room temperature. The dispersions were cooled down to 4^0C in refrigerator; the Poloxamer was added slowly with continuous stirring. The dispersions were then stored in a refrigerator for overnight until clear solutions were obtained. Finally, volume was adjusted with distilled water. All final formulations were evaluated for their clarity, pH, content uniformity, gelation temperature, mucoadhesive strength, viscosity study and diffusion study. Compositions of various prepared formulations are given in following table [7, 8].

Table no. 1: Composition of thermoreversible Salbutamol Sulphate gel

Batch code	Poloxamer 407 (%w/v)	DRG (%w/v)	CMDRG (%w/v)	Salbutamol Sulphate (%w/v)	Benzalkonium Chloride (%w/v)	Distilled Water
A	15	0.5	_	0.25	0.02	Q.S.
B	15	_	0.5	0.25	0.02	Q.S.
C	15	1	_	0.25	0.02	Q.S.
D	15	_	1	0.25	0.02	Q.S.
E	16	0.5	_	0.25	0.02	Q.S.
F	16	_	0.5	0.25	0.02	Q.S.
G	16	1	_	0.25	0.02	Q.S.
H	16	_	1	0.25	0.02	Q.S.

Evaluation Of Formulations

Infrared Spectroscopy:

FTIR absorption spectrum of drug, polymer and physical mixture of drug and polymers were recorded by potassium bromide dispersion technique using Schimatzu FTIR spectrophotometer (SHIMADZU). Where in 1-2 mg of drug sample and potassium bromide was mixed uniformly and powder blend was compressed into discs by applying a pressure of 5 tons for 5 minutes in a hydraulic press and the spectrum was recorded [9].

Differential Scanning Calorimetry:

DSC study was performed for drug, polymer and physical mixture of drug and polymers. The drug sample (1 mg) was sealed in aluminum pan and heated over a temperature range 30 to 350°C using PERKIN ELMER 4000, instrument under nitrogen purging at flow rate 20 ml/min [9, 10].

Clarity:

The clarity of various formulations was determined by visual inspection against black and white background and it was graded as follows; turbid: +, clear: ++ and very clear: +++ [8].

pH:

pH of each formulation was measured using pH meter when they are in sol condition [8].

Content uniformity:

Test for content uniformity was carried out for all the prepared gel formulations. The vials (n=3) containing formulation were properly shaken for 2-3 min. 0.1 ml from each formulation was taken in 50 ml volumetric flask, dissolved in phosphate buffer solution pH 6.8 with gentle stirring and final volume was adjusted. 1 ml from this solution was diluted up to 100 ml with phosphate buffer solution pH 6.8 to obtain concentration 2µg/ml respectively. The absorbance was measured at analytical wavelength 276 nm using phosphate buffer solution pH 6.8 as blank using Bioera Elite UV spectrophotometer [11].

Gelation temperature:

Gelation temperature of each formulation was determined by visual inspection method as described in preformulation study. Measurements were carried in triplicates for each formulation [12].

Determination of mucoadhesive strength:

Nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughter house. Tissues were immediately used after separation. This section of nasal tissue instantly fixed with mucosal side out on to glass slide using rubber band. The slide with nasal mucosa was stored at 37^0C for 5 minutes. Then this glass slide was connected to the right arm of the balance in inverted position. The nasal mucosa was hydrated with distilled water prior to mucoadhesion testing. The slide with nasal mucosa fixed to height adjustable stand. According to previously reported methods. The Mucoadhesive force of the formulation under study was determined by measuring the force required to detach the formulation from a sheep nasal mucosa using the measuring device. The fixed amount of each gel formulations were placed on the lower slide with nasal mucosa. The lower slide was then elevated till the surface of the sample came in contact with the nasal mucosa. Both the in-situ gel and the hydrated nasal mucosa were left in contact for 2 min using a preload of 10g to establish the contact between them and allow the formation of an adhesive bond. The preload time and force were kept constant for all the tested formulations. The nasal mucosa was detached from the tested sample and the weight required to detach the tested sample from the nasal mucosa was calculated by difference. The results were the mean of three runs. The detachment force (dyne/cm2) was determined using the following equation

Detachment force (dyne/cm^2) = mg /A

Where, m is the weight of water in grams;

g is acceleration due to gravity taken as 980 cm/sec^2;

And A is the area of the mucosa (area of contact) and is equal to πr^2 (r is the radius of the mucosa).

Measurement was carried in triplicates for each formulation [7, 13].

Syringeability:

All formulations were allowed to pass from syringe and checked whether it passes or not through the syringe [14].

Viscosity study:

The viscosities of various formulations were determined by using Cone and Plate viscometer (Brookfield viscometer Model Cap 2000). Required quantity of formulation was applied on lower plate of the viscometer using micropipette. The temperature was increased in steps of 2^0C/minute, from 28^0C to 36^0C. The viscosity was measured as a function of the temperature (^{0}C) [11].

Ex-vivo diffusion study:

Ex-vivo diffusion study was carried for all batches. Fresh nasal tissue was carefully removed from the nasal cavity of sheep obtained from the local slaughter house. The mucosa was stored in normal saline after removal of blood and bony cartilage from mucosal membrane it becomes ready to use. Tissue sample was tied between the receiver compartment and donor compartment. The position of the donor compartment was adjusted so that membrane just touches the diffusion medium. 30 ml of 6.8 pH phosphate buffer solution was added to the acceptor chamber and agitated with magnetic stirrer at 37^0 C. After pre incubation time of 20 min, formulation equivalent to 0.25%w/v of Salbutamol sulphate was placed in the donor chamber. From the acceptor compartment 1 ml sample aliquots were withdrawn at predetermined time interval up to 8 hrs replacing the sample volume with 6.8 pH PBS after each sampling, filtered and analysed by UV spectrometer at 276 nm [11, 7].

Drug Release Kinetics:

Drug release kinetics was obtained by the best curve fitting method and comparing the correlation coefficients of release data with zero order, first order, Higuchi model and Korsemeyer-Peppas models. The ex-vivo drug release profile of all gel formulations were fitted on to various kinetic models in order to find out the mechanism of drug release.
The best fit model with highest correlation coefficients was selected [15].

RESULTS

FTIR Spectroscopy

1. Salbutamol Sulphate

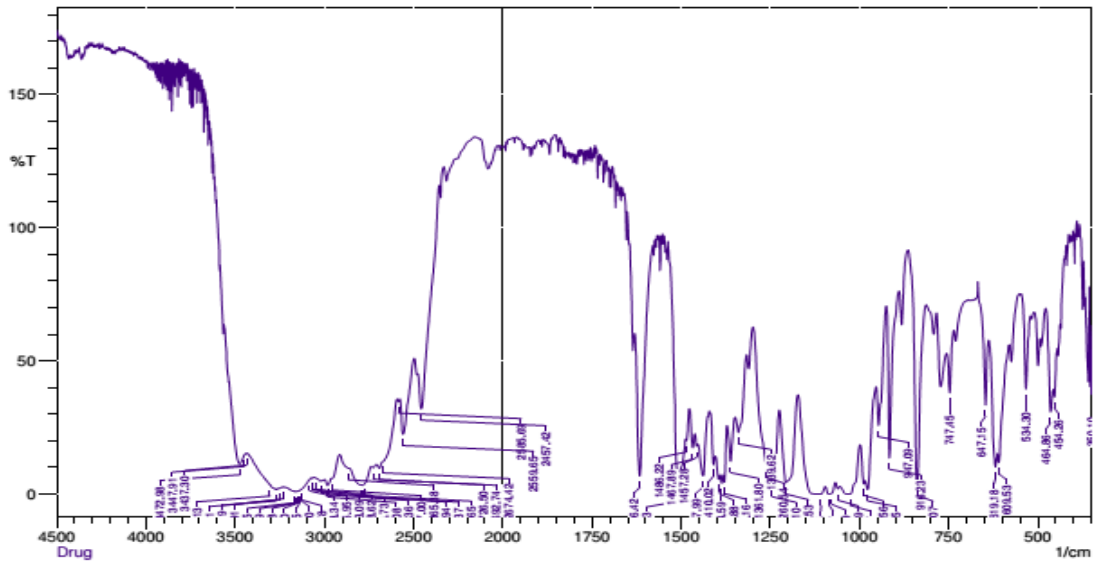

Figure 1: FTIR spectrum of Salbutamol Sulphate

FTIR absorption spectrum of Salbutamol Sulphate was recorded by potassium bromide dispersion technique using SCHIMATZU FTIR spectrophotometer as shown in Figure 1. Characteristic peaks at 839.07, 1486.22, 1616.12, 2982.08, 3271.41, 3472.98 cm^{-1} corresponds to secondary amine, C-C stretching, C-C stretching aromatic, C-H stretching, O-H stretching, O-H Phenol respectively were observed.

2. Poloxamer 407:

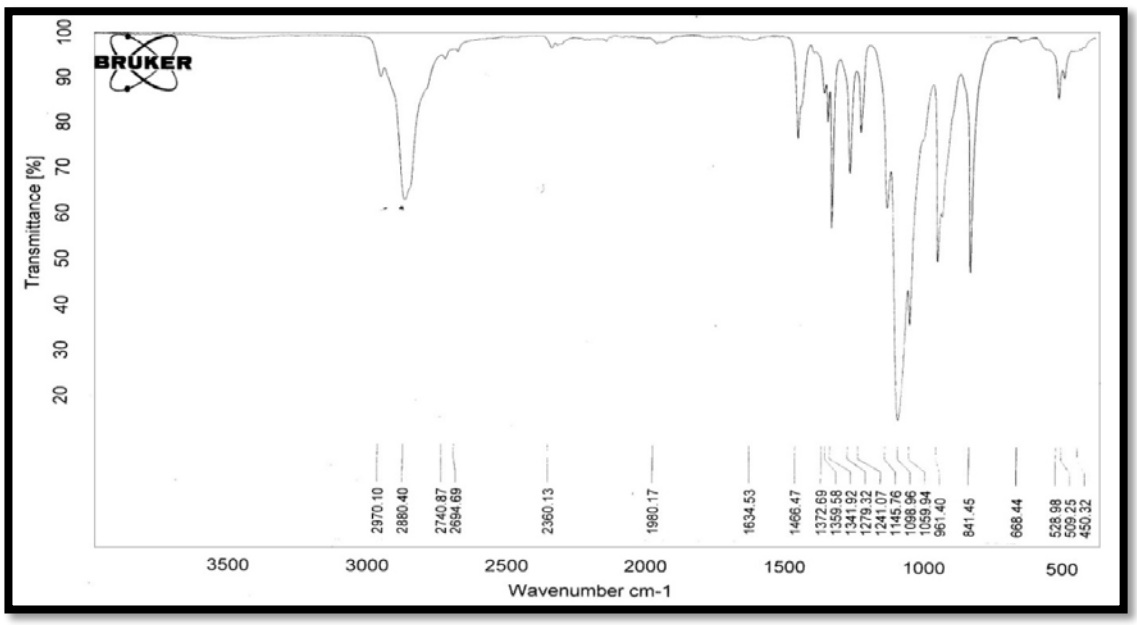

Figure 2: FTIR spectrum of Poloxamers 407

In FTIR spectra for Poloxamer, the characteristics peak at 2970.10 cm^{-1} is due to C-H alkane, 2880 cm^{-1} due to C-H branched alkane, 2740.87cm^{-1} due to O-H stretching, 1059.94 cm^{-1} due to C-O-C ether were observed.

3. Delonix regia Gum (DRG)

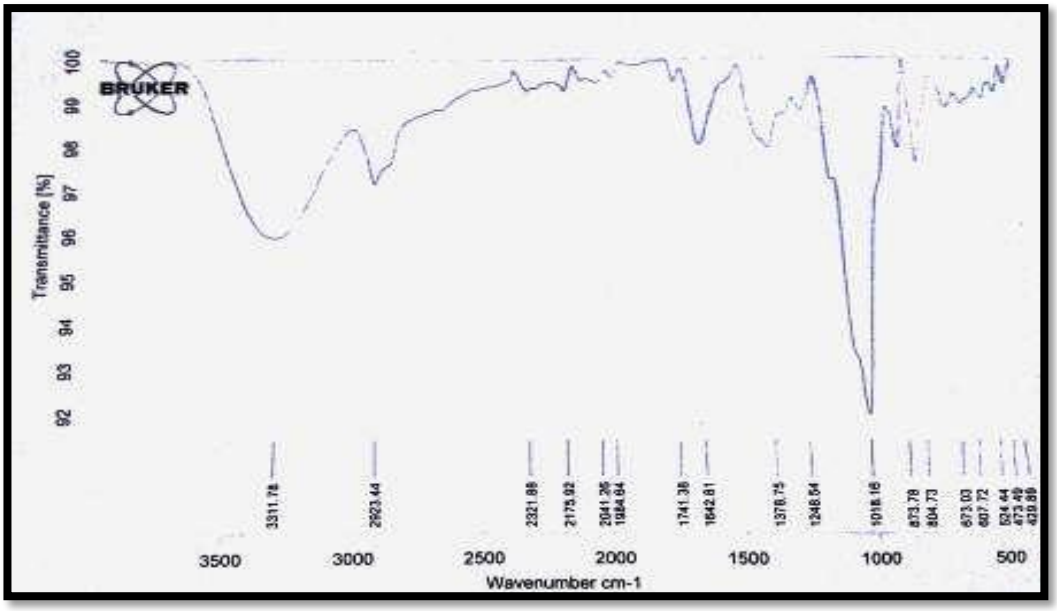

Figure 3: FTIR spectrum of DRG

In FTIR spectra for DRG, the characteristics peak at 3311.78cm^{-1} due to O-H stretching vibration, 2923.44cm^{-1} due to CH stretching of CH_2 group, 1016.92 due to C-O stretching 1741.38 cm^{-1} may be due to ring structure of mannose and galactose were observed.

4. Carboxymethylated Delonix Regia Gum (CMDRG)

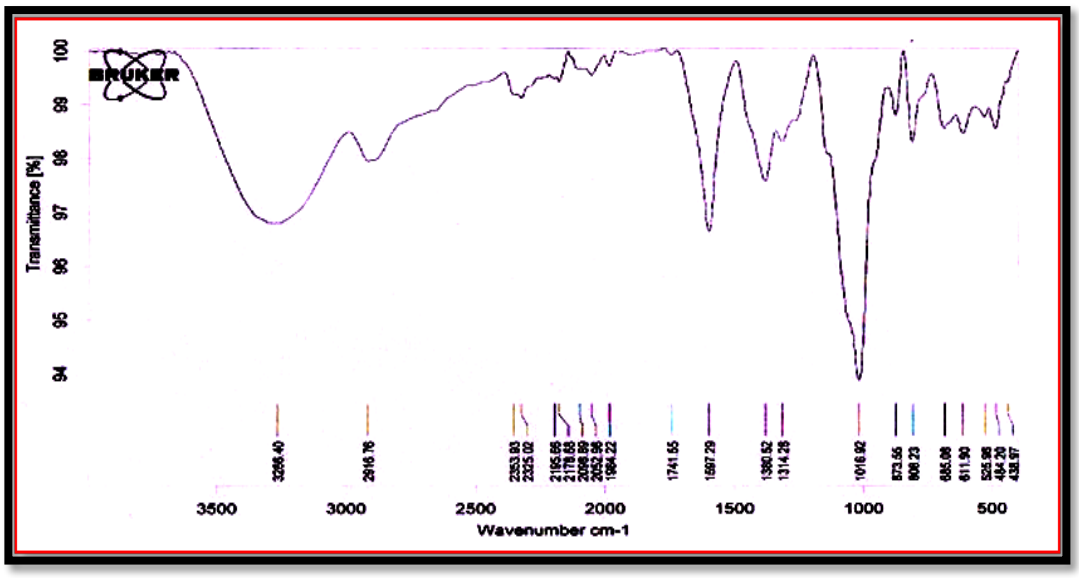

Figure 4: FTIR spectrum of CMDRG

FTIR spectra for CMDRG showed a strong intensity peak at 1597.29 cm^{-1} corresponds to asymmetric carboxylate anions. Peak at 1380.52 cm^{-1} are assigned to methyl (-CH_2) group. This confirmed incorporation of carboxymethyl groups into the DRG molecule

5. Physical Mixture of Drug, Poloxamer and DRG

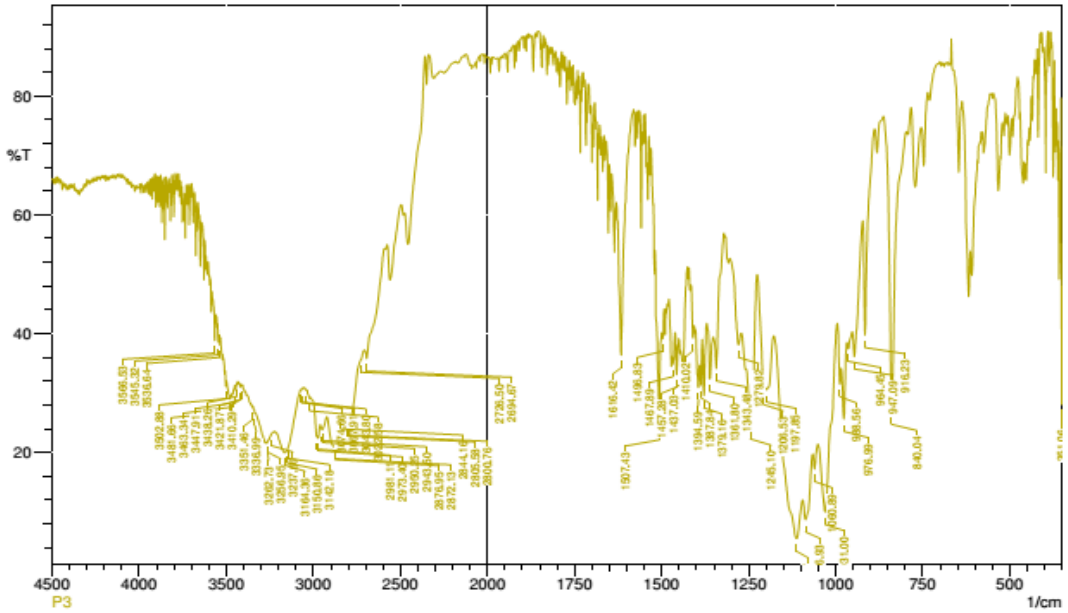

Figure 5: FTIR spectrum for physical Mixture of Drug, Poloxamer, and DRG

6. Physical Mixture of Drug, Poloxamer and CMDRG

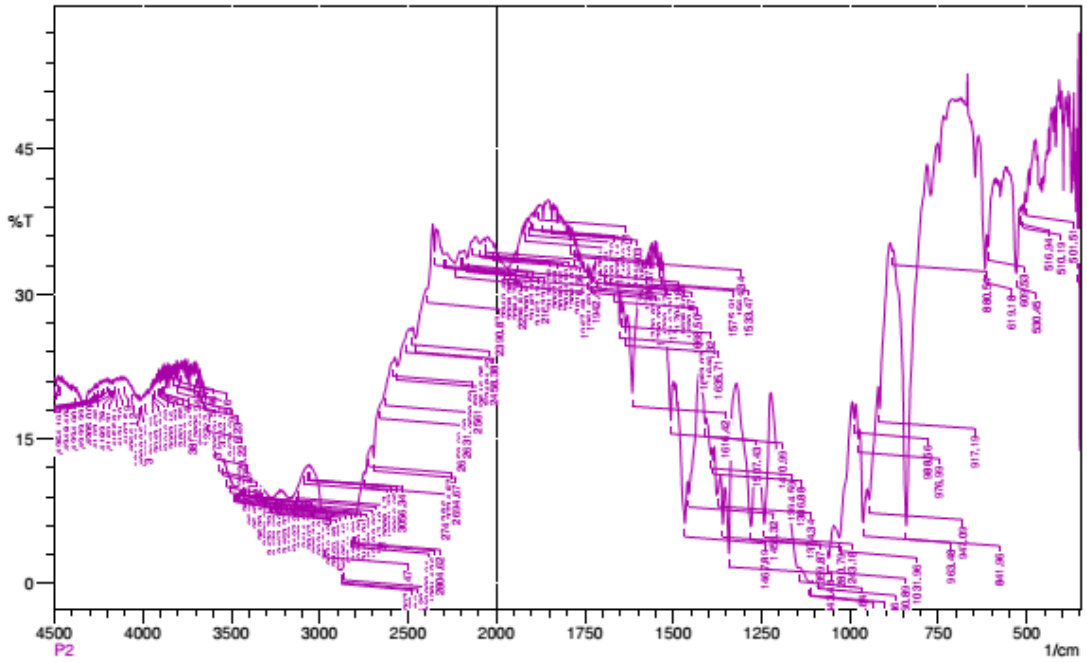

Figure 6: FTIR spectrum for physical Mixture of Drug, Poloxamer, and CMDRG

From FTIR spectra it was observed that there are no significant changes in the position of the characteristic peaks of drug when mixed with Poloxamer, DRG and CMDRG which indicates compatibility of polymers with drug.

Clarity:

Formulations containing 0.5 and 1 % DRG were found clear while that containing 0.5 and 1% of CMDRG were found very clear as shown in Table no. 2.

pH:

pH of all formulations were in the range of 5.3 ± 0.173 and 6.5 ± 0.057 as shown in Table no. 2. To avoid nasal irritation, formulation pH should be between 4.5 and 6.5.

Content uniformity:

The percentage drug content of all formulations found to be in the range of 93.6 ± 0.8 to 98.66 ± 0.923 % w/v. The difference in drug content of each batch may be due to dilution error arises during the determination of drug content.

Gelation temperature:

The gelation temperature (T_g ^{0} C) of formulations were found within the 32 ± 1 to 39 ± 0.577^{0}C which is shown in Table no. 2.

Mucoadhesive strength:

It is very important that the nasal gel formulation must have suitable gel strength. The stronger the bioadhesive force greater will be the nasal contact time. As the concentrations of polymers increased the mucoadhesion force was also increased. Results are presented in Table no. 2 and Figure 7.

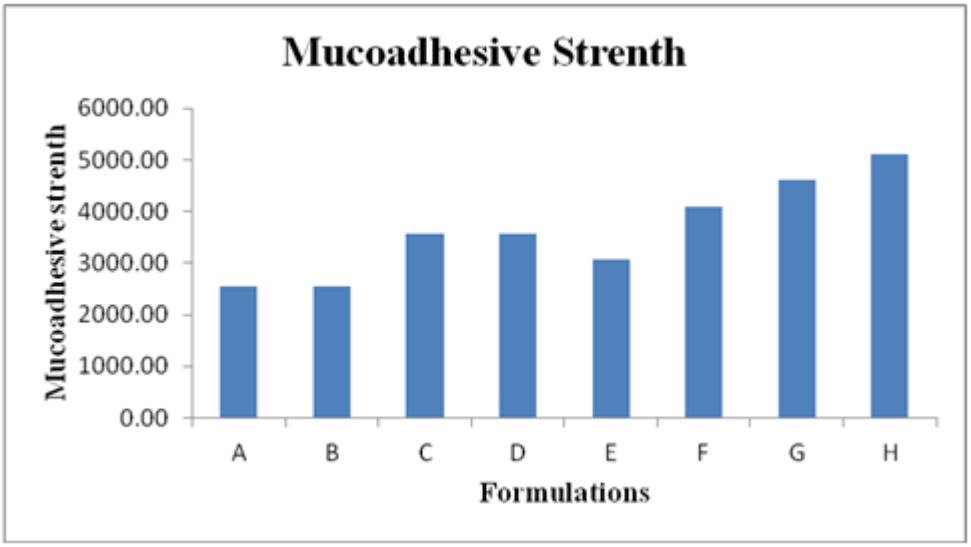

Figure 7: Mucoadhesive Strength of thermoreversible gel.

Table no. 2: Results of clarity, pH, gelation temperature (Tg), drug content of formulations and mucoadhesive strength (Fm).

Batch Code	Clarity	pH n=3	T_g(^{0}C) n=3	F_m (dyne /cm^2) n=3	Drug content (% w/v) n=3
A	++	6 ±0.057	39 ± 0.577	2557.18 ± 0.16	97.33 ± 0.92
B	+++	6.2 ± 0.11	38 ± 1	2557.25 ± 0.04	96.8 ± 0.8
C	++	6.5 ±0.05	37 ± 0.57	3579.83 ± 0.30	97.06 ± 0.46
D	+++	5.9 ± 0.11	36 ± 0.57	3589.56 ± 0.35	93.6 ± 0.8
E	++	5.9 ± 0.23	36 ± 0.57	3068.90 ± 0.90	97.33 ± 0.46
F	+++	5.9 ± 0.28	35 ±1.52	4090.56 ± 0.38	96.53 ± 1.22
G	++	6 ± 0.11	35 ±1.52	4602.45 ± 0.42	98.66 ± 0.92
H	+++	5.3 ±0.17	32 ± 1	5112.49 ± 0.44	96.8 ± 1.38

Syringeability:

All formulations passed from the syringe.

Viscosity study:

Viscosity measurement of each formulation at various temperatures, showed that there was increased in viscosity with increased in the concentration of poloxamer were represented in Figure 8.

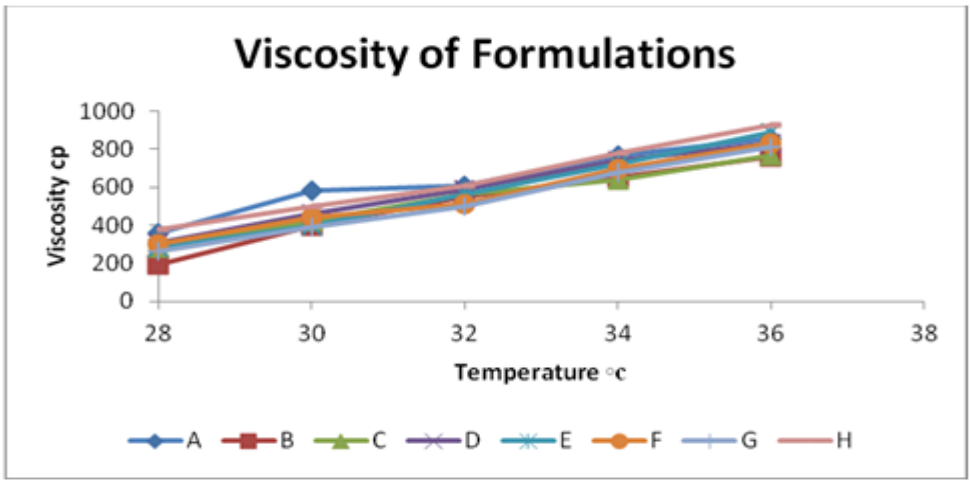

Figure 8: Viscosity of Formulation.

Ex-vivo diffusion study:

Ex vivo diffusion study was carried out by using nasal mucosa of goat. Permeation profile of drug was shown in Figure 9,10,11,12

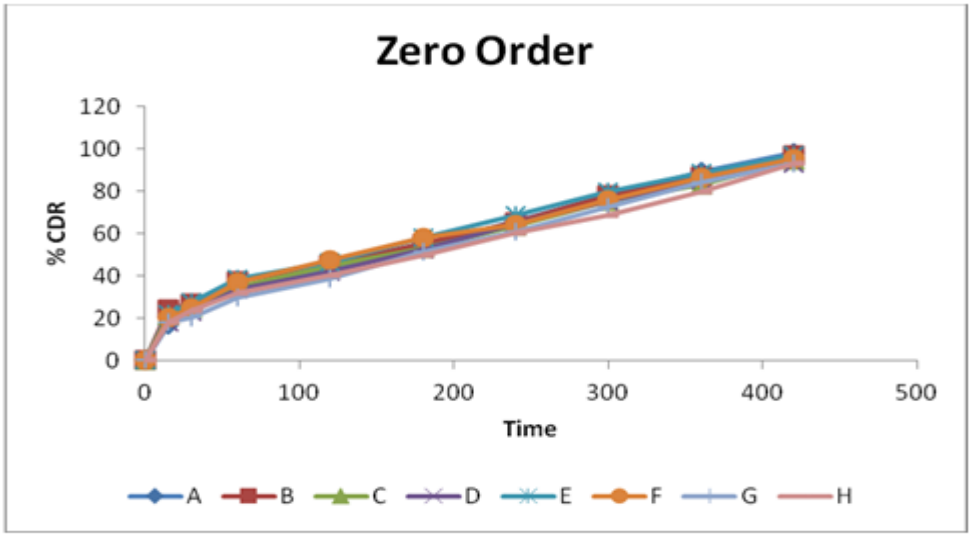

Figure 9: Zero-order plot for different gel formulations

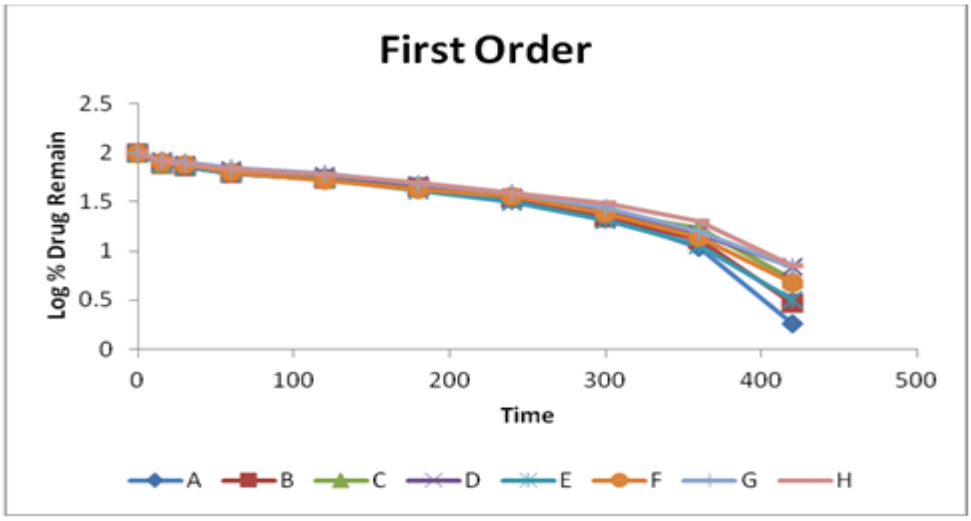

Figure 10: First order plot for different gel formulations

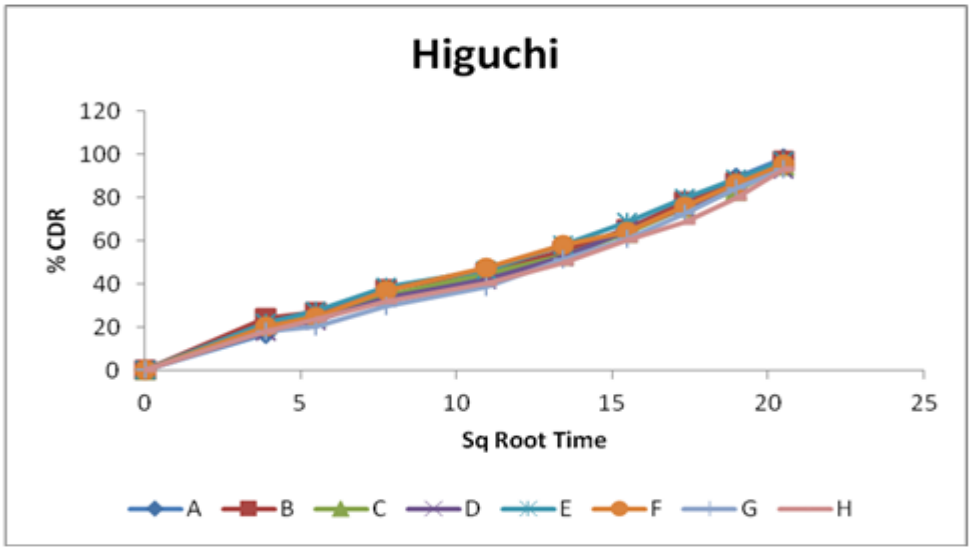

Figure 11: Higuchi plot for different gel formulations

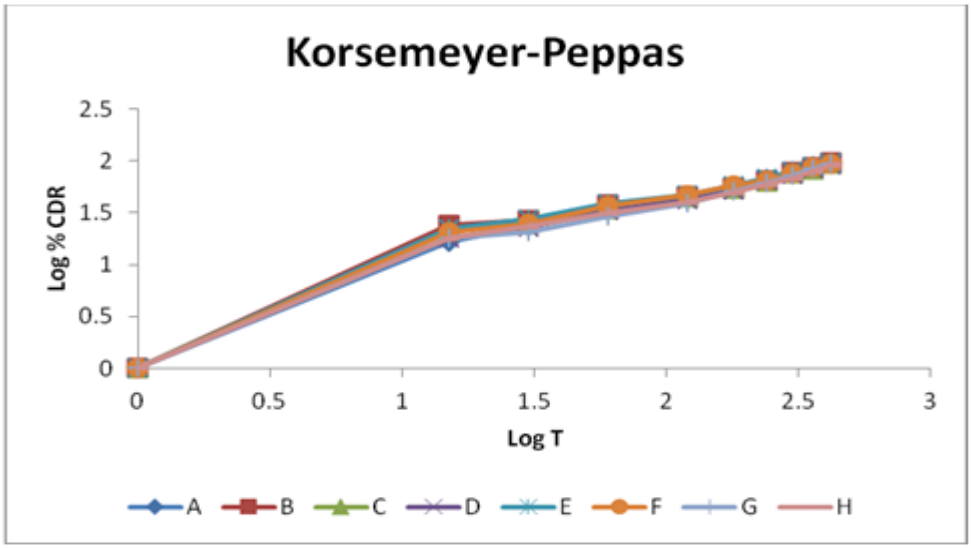

Figure 12: Korsemeyer-Peppas plot for different gel formulations

Table no. 3: Various parameters of the model equations of the Ex-vivo release kinetics

Formulation	Zero Order	First Order	Higuchi model		Korsemeyer-Peppas model	
	R^2	R^2	R^2	K	R^2	n
A	0.967	0.843	0.987	4.661	0.955	0.765
B	0.947	0.869	0.984	4.397	0.915	0.762
C	0.954	0.899	0.987	4.32	0.928	0.757
D	0.962	0.944	0.989	4.421	0.947	0.756
E	0.943	0.902	0.992	4.496	0.928	0.774
F	0.945	0.911	0.992	4.417	0.943	0.776
G	0.974	0.923	0.981	4.394	0.946	0.742
H	0.963	0.894	0.98	4.192	0.941	0.717

R^2 = correlation coefficient, K = release rate constant, n = release exponent of Korsemeyer-Peppas model.

Ex-vivo drug release study was performed on goat nasal mucosa. From % cumulative drug release data, it was observed that increase in concentration of mucoadhesive polymers produced decrease in drug release rate. Also it was observed that drug release rate was reduced with increasing poloxamer concentration.

CMDRG showed marked decrease in drug release rate as compare to DRG when both were used in same concentrations. This might be due to increase in mucoadhesive strength of CMDRG as well as may be due to increase in viscosity of formulations with increase concentration of polymers.

Drug Release Kinetics:

The ex-vivo drug release profile obtained from diffusion formulations were fitted to zero order, first order, Higuchi and Korsemeyer-Peppas model as shown in Table no.3.

The best fit model was selected from highest correlation coefficients. Thus the best fit model was Higuchi model.

The data obtained was put in Korsemeyer-Peppas model to find out 'n' value which describes the drug release mechanism.

If the value of n<0.5, the drug release is diffusion controlled. If n<1.0, the drug release is swelling controlled.

All formulations showed the value of n between 0.5 to 1.0 as shown in Table no. 3 which shows anomalous type of release mechanism.

CONCLUSION

Natural polymer and its carboxymethyl derivative were used to enhance mucoadhesion and poloxamer 407 was used for its sol gel property. DRG was successfully derivatized to CMDRG by etherification reaction. Drug and polymers were characterized by FTIR and DSC analysis. Salbutamol Sulphate was selected for formulation of in situ gel. DRG and CMDRG were used in varying concentration as a mucoadhesive polymer. Increase in concentration of polymers should decrease in gelation temperature of formulations while increase in concentration of polymers produced increase in mucoadhesion force of all formulations. Delonix Regia Gum and its carboxymethyl derivative can be successfully used as mucoadhesive polymer in a concentration range 0.5 to 1% along with poloxamer 407 (15 to 16%) in the formulation of in situ nasal gel of Salbutamol Sulphate.

From the results it was concluded that, nasal gel of Salbutamol Sulphate showed better drug absorption by avoiding first pass metabolism.

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Correspondence Author:

Shakir Shoukat Sikilkar*

M.Pharmacy ,Svpm's College Of Pharmacy, Malegaon (Bk), Baramati, Pune, 411315.

Correspondence Author email: shakir.sikilkar9@gmail.com

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