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Formulation and evaluation of *in-situ* gel of bromhexine hydrochloride for nasal delivery

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ABSTRACT

The aim of the present study was to minimize the unwanted toxic effects of Bromhexine hydrochloride (BHC) by kinetic control of drug release. BHC was incorporated into the, blends of bio compatible, bio polymers such as Poloxamer (PLX) and Hydroxy Propyl Methyl Cellulose (HPMC) in the form of in situ gel by cold technique to reduce muco ciliary clearance by using mucoadhesive polymer in gel, thereby, increasing the contact of formulation with nasal mucosa and hence improving the absorption of drug. The results revealed that increased bio adhesive polymer HPMC concentration, decrease the gelation temperature(T1) and gel melting temperature (T2). pH of all the formulations were found to be within the range between 6.9- 7.5 ,and the nasal mucosa can tolerate the pH of the above solutions. The drug content for all the prepared formulations was found to be in the range of 96%- 100%. The result of mucoadhesion test indicate that the level of HPMC increases, the mucoadhesive strength also increases. The developed formulations had optimum viscosity required for convenience in application at the site. With increase in the concentration of HPMC increase in viscosity was observed and influences diffusion of drug particles. The optimized formulations shows the controlled drug release (85.12%) than aqueous drug solution(92.32%). From the in vitro permeation study viscosity of the formulation is increased and release of the incorporated drug can be prolonged. The drug release performance was greatly effected by bio polymers used and their compositions in the in situ gels preparation, which allows absorption in nasal mucosa.

Key Words: *In situ* gel, Bromhexine Hydrochloride, HPMC, Poloxamer407, Nasal delivery,

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INTRODUCTION

Bromhexine hydrochloride (2-amino-3,5-dibromobenzyl (cyclohexyl) methyl ammonium chloride) is an oral mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus [1]. It acts by disrupting the structure of acid mucopolysaccharide fibers in mucoid sputum and produces a less viscous mucus, which is easier to expectorate. Even though the oral absorption is greater than 90%, due to its extensive first pass metabolism, it has a very low bioavailability of 20%. They were also found to precipitate gastro intestinal ulceration. Hence, these drugs cannot be given by oral route [2]. Therefore, an intranasal delivery system of Bromhexine Hydrochloride was preferred.

Nasal route has been selected because, nasal mucosa has a rich supply of blood, large surface area and porus endothelial membrane .Nasal delivery of drugs offers many advantages over other delivery routes. To improve nasal absorption of drugs it is necessary to enhance the nasal residence time of drug by including bio adhesive in the

formulation [3].HPMC is used as a bio adhesive polymer in the present study to formulate mucoadhesive drug delivery system through nasal mucosa [4]. HPMC is bioadhesive polymer which is nonionic, a good carrier material for pharmaceutical application, can accommodate high levels of drug loading, non toxic and exhibits high swelling capacity. When HPMC contact with water hydrates rapidly, leading to a transition from the glassy to the rubbery state, which results in the formation of a gel layer with a significant effect on release kinetics of the incorporated drug .Moreover, they are surface active and hence provide excellent film formation [5,6].

In the present study our intention is to achieve controlled drug absorption and prevent GI ulceration. The nasal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption. This is due to the large surface area, porous endothelial membrane, and high total blood flow [7]. Also, pharmacokinetics shows, intranasal administration circumvent first-pass elimination. Recently, focus has been made on the nasal mucosa as an alternate route to achieve faster and higher drug absorption .The nasal cavity offers a number of unique advantages such as easy accessibility, good permeability especially for lipophilic, low molecular weight drugs, hepatic first pass metabolism and potentiates direct delivery to the brain [8].

Nagesh C et al., [9] had prepared novel in situ gallen gum based ophthalmic drug delivery system of ciprofloxacin hcl and dexamethasone. All the formulations were sterilized in an autoclave at 121°C for 15mins. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, in vitro diffusion study and ICH stability studies. The developed formulations exhibited sustained release of drug over a period of 7 hours thus increasing residence time of the drug and optimized formulations also found satisfactorily stable, thus these in situ gelling systems may be a valuable alternative to the conventional systems.

Nagargoje et al., [10] developed ophthalmic delivery system of an antifungal agent, fluconazole, based on the concept of ion-activated in situ gelation. It was reported that ocular in situ gels can increase the drug residence time thus increasing the bioavailability. Gelrite was used as the gelling agent in combination with HPMC E-50(Hydroxy Propyl methyl Cellulose) that acted as a viscosity-enhancing agent. Study results revealed that the formulations were therapeutically efficacious, stable and provide sustained release of drug over a period of 8 Hrs. It was found that developed system is a best alternative to conventional ophthalmic drops.

Gowda et al., [11] developed and evaluated a mucoadhesive in-situ gel; formulation was developed to have a controlled kinetic drug release and to minimize the toxic effects of diltiazem Hydrochloride (DTZ). DTZ was incorporated into the, blends of thermoreversible, bio adhesive polymers such as poloxamer (PLX) and Hydroxy Propyl Methyl cellulose (HPMC) in the form of in-situ gel by cold technique to reduce mucociliary clearance, and thereby it will increase the contact of formulation with nasal mucosa and hence improving the absorption of drug. The results revealed that as the increase of bio adhesive polymer HPMC concentration, decrease in the gelation temperature (T1) and increase in gel melting temperature (T2).

Though nasal delivery was proved to be useful it still has some limitations like muco ciliary clearance which reduces the absorption of drugs. In this regard it is important to increase the residence time of the drug on the nasal mucosa. By using bioadhesive polymers we can increase the residence time of drug in the nasal cavity. Pluronic F 127 is the reversible triblock copolymer used which forms clear thermo reversible gel at high concentrations. The concentrated solutions are transformed to low viscosity transparent solutions at 5^0 C and to solid gel on heating to body temperature [12]. Pluronic F-127 (Poloxamer 407, PF-127)is a thermoreversible gel [13]. This characteristic has allowed PF-127 to be used as a carrier for most routes of administration including oral, topical [14] ,intranasal [15], vaginal, rectal [16], ocular [17], and parenteral routes [18] .The potential use of PF-127 as an artificial skin has also been reported

The most prominent advantage of a in situ gel over normal gel is that it is fluid like prior to contact with nasal mucosa. Hence, there is convenience of administration for patients and also accurate drug dosing. The objective of the present study is to develop in situ gel of Bromhexine Hydrochloride, characterize and study the drug release properties with favorable gelation, rheological and release property. This may give patient friendly, needle free dosage form.

MATERIALS AND METHODS

Materials:
Bromhexine Hydrochloride was obtained as a gift sample from MicroLabs , Bengaluru, India. Pluronic F 127 , Hydroxy Propyl Methyl Cellulose and all the other reagents used were of analytical grade procured from Loba Chemicals, Mumbai, India.

Preparation Of *in situ* gels:
Pluronic gels were prepared by cold technique [19]. The formulation ratio presented in Table 1. To the 1%w/v, solution of drug in distilled water, HPMC was added in the concentrations of 0.2%, 0.4%, 0.6% ,0 .8%, 1%W/V and the above mixture was stirred until HPMC completely dissolves. To the above mixture, PLX was added (19%w/v used as base) and the formulations were kept at 4^0C overnight until clear gel was obtained.

Physicochemical Studies
Fourier transformation infrared spectroscopy (FT-IR)
FTIR spectra of pure drug, physical mixture (BHC + PLX + HPMC) and physical mixture with out drug (PLX + HPMC) were obtained using KBr pellet method (applying 6000 kg/cm ^{2}). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, Model 8033,USA). Each spectrum was recorded in the frequency range of 4000-450 $cm^{-1.}$.

X-ray diffractometry
The X-ray diffractograms of BHC and Physical mixture (BHC + PLX+HPMC) were recorded by using Philips XuPert MPD (Netherland) diffractometer with tube anode Cu over the interval 4-40^o/2θ. The generator tension (voltage) 40kV, generator current 45 mA and scanning speed 2^o/min.

EVALUATION OF GELS
Measurement of Gelation Time:
A 2ml aliquot of gel was taken in a test tube and kept in an oven maintained at 37^0C. The sample was examined for gelation.

Measurement of Gelation temperature (T1) and Gel Melting Temperature(T2):
Gelation temperature and gel melting temperature were determined by Miller and Donovan technique [15]. 2 ml aliquot of gel was taken in a test tube and placed in water bath. The temperature of the water bath was increased slowly(0.5^0C each time) and the gel was allowed to equilibrate at each new setting for 5 minutes .The sample was then observed for gelation. If gelation has occurred the meniscus would no longer move upon tilting to $90^{0.}$ This temperature was noted down as T1. If the above formed gel is further heated, it results in the formation of a viscous liquid and starts flowing. This temperature is the gel melting temperature was noted down as T2. If gel melting has occurred, gel starts flowing upon tilting the test tube to 90^0C.

pH of Formulation :
1 ml of each formulation and sufficient amount of distilled water was added to make up the volume to 25 ml. pH of the resulting solution was determined using digital pH meter.

Drug Content:
1 ml of each formulation was taken in a 10 ml volumetric flask and diluted with methanol to make up the volume to 10 ml. From this solution, 1 ml was taken and further diluted to 10 ml. Absorbance of the prepared solution was measured at 255.4 nm by using UV Visible spectrophotometer(Shimadzu UV-1800).

Determination of Mucoadhesive Strength:
The mucoadhesive strength was determined by using the modified method [20]. The force required to detach the formulation from nasal mucosal tissue was determined to find out mucoadhesive potential of each formulation. For this purpose, a section of sheep nasal mucosa 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 gel formulation between them held in contact with each other, for 2min 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.

Mucoadhesive Strength (dynes/cm ^{2}) = mg/A, (1)

Where, m = weight required for detachment in gram,
g = Acceleration due to gravity (980cm/s2),
A = Area of mucosa exposed.
The nasal mucosa was changed for each measurement.

Viscosity Measurement:
The viscosity measurements were carried out by using Brookfield programmable DV-II LV model (Brookfield Eng.Lab.,Inc.USA). The gel sample was placed in small sample adapter. Temperature was increased in the range of 20^0C to 40^0C, using water circulation jacket. The temperature sensing probe was lowered in gel and temperature of gel was recorded. Viscosity at various temperatures was recorded.

***In-vitro* Release Studies:**
Drug release from *in situ* gel was carried by nasal diffusion cell, using cellophane membrane (mol.wt.12, 000-14,000) with permeation area of 0.8cm^2. 60ml of phosphate buffer pH 6.4 was placed in the acceptor chamber and gel containing drug equivalent to 10mg was placed in donor chamber. At predetermined time points, 1ml sample was withdrawn from the acceptor compartment with continuously replacing by fresh buffer, (pH 6.4 phosphate buffer) for a period of 5 h. The samples were suitably diluted and measured spectrophotometrically at 255.4 nm. The concentration of drug was determined from a previously constructed calibration curve.

***In –vitro* Permeation Study:**
Fresh nasal tissue was removed from nasal cavity of sheep obtained from local slaughter house. Tissue was inserted in the nasal diffusion cell with permeation area of 0.8 cm^2. Gel containing drug equivalent to 10mg was kept in donor compartment. At predetermined time point sampling was done. Blank samples (without drug) were run simultaneously throughout the experiment. Amount of drug permeated was determined by UV spectrophotometry at 255.4 nm.

Differential Scanning Calorimetry (DSC) studies
DSC study was carried out using DSC-60 instrument (M/s Shimadzu) to check the matrix formation as well as the compatibility of ingredients. DSC thermograms of pure drugs (BHC) and formulation were taken for their identical endothermic reaction. Further their physical mixtures of drugs and polymers were also studied for their interactions**.**

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy
The FT-IR spectra of BHC and physical mixture (formulation F2) samples are shown in Fig 1. The FT-IR spectrum of bromhexine hydrochloride showed absorption bands at 3441, and 3301cm^{-1}in the region of 3500-3100 cm^{-1} for the NH-stretching vibration of Ar-NH2 and sharp bands at 1634 and 1483 cm^{-1} in the region of 1700- 1400 cm^{-1} for the NH-bending vibration of the Ar-NH2. The FTIR spectrum of physical mixture was similar to the spectra of BHC. The characteristic IR absorption peaks of BHC compared with the IR spectra of BHC in physical mixture (formulation F2), were not alter, indicating no chemical interactions between the drug and polymers used.

X-ray diffractograms
The X-ray diffractograms of BHC and physical mixture (formulation F2) are presented in Fig.2. The pure BHC showed several diffraction peaks, exhibiting a main sharp peak at 7.2, 22.3 and 35.3^o (2Θ) and secondary peaks at 13.8, 14.9, 17.8^o(2 Θ). No diffraction peak was observed in the PLX and HPMC diffractogram. The diffraction patterns of physical mixture (formulation F2) exhibiting a main sharp peak at 7.3, 22.4 and 35.2^o (2Θ) and secondary peaks at 13.7, 14.7, 17.9 ^{o}(2). The several sharp diffraction peaks from BHC diffractogram exhibited that the drug BHC is in crystalline form. The diffraction patterns of physical mixture (formulation F2) corresponded to the superimposition of BHC indicated that the crystallinity of BHC was not changed. The fine crystals of BHC either dispersed on to the surface or cavities of PLX and HPMC.

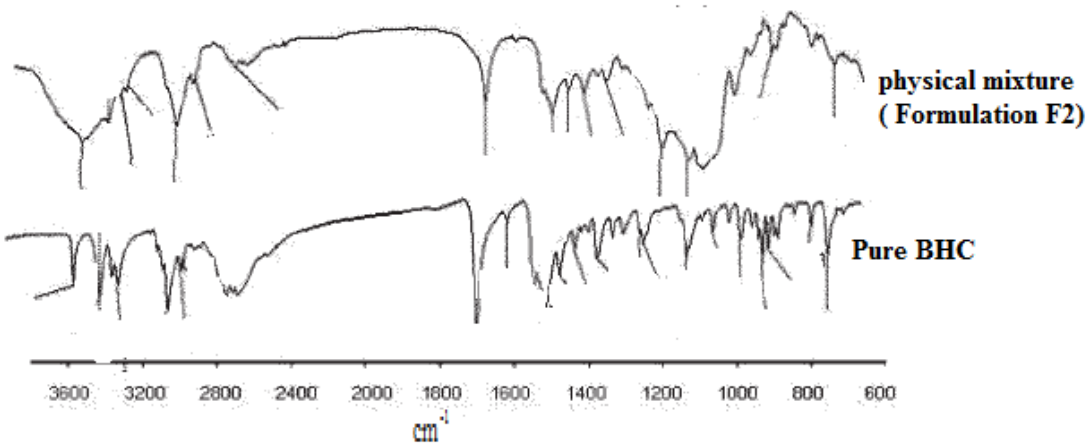

Fig 1: FT – IR spectra of BHC and physical mixture (Formulation F2)
BHC = Bromhexine hydrochloride

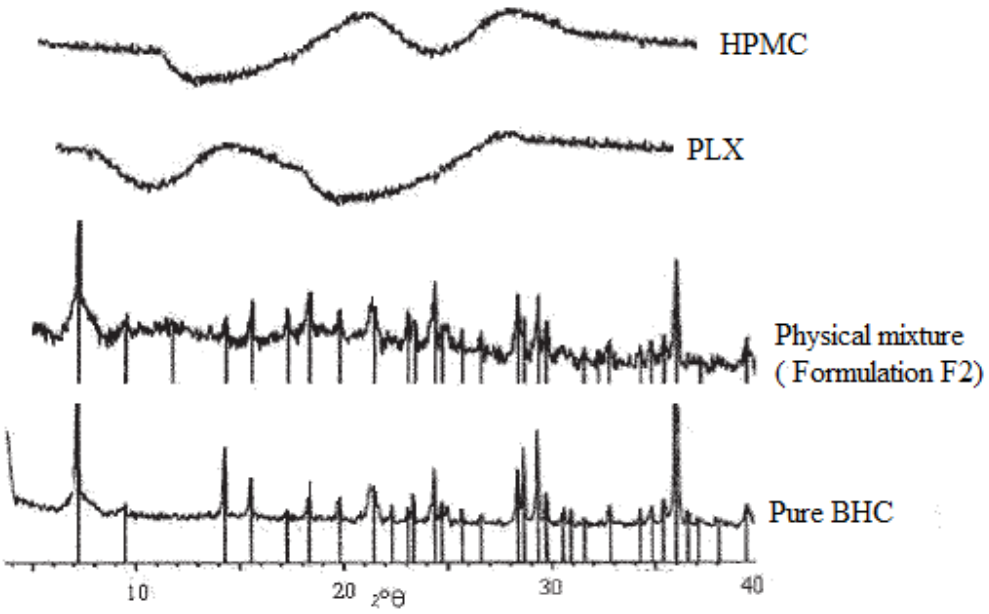

Fig 2: X – Ray diffractograms of BHC, Physical mixture (Formulation F2), PLX and HPMC.
BHC = Bromhexine hydrochloride, PLX = Poloxamer and HPMC = Hydroxy Propyl Methyl Cellulose

Evidence have shown in the recent years that bio adhesive polymers have the physical properties and behavior suitable to prepare bio compatible bio degradable in situ gels to release the drug in the nasal mucosa [21, 22]. In the present study, the modified novel cold technique was employed using bio adhesive polymers and non toxic solvents to prepare the in situ gels. The present method is quite different from that reported by Schmolka [15] because in the present study, HPMC was used as a bioadhesive polymer (0.2- 1% w/v), PLX as base (19% w/v) at various concentration to study the gelation temperature, mucoadhesive strength, viscosity and % cumulative drug release.

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 formulations the gelation time was found to be with in 2 minutes. PLX 19% w/v was used to produce the *in situ* gels with in 2 min. The resultant *in situ* gel did not have any surface irregularities.

Gelation Temperature (T1) and Gel melting Temperature (T2):
At gelation temperature, liquid phase makes transition into gel. Due to the addition of HPMC and BHC there is a change in T1 of gel formation. Study shows that formulation F1 having gelation temperature of 31^0C (low level of HPMC-0.2%w/v) where as F2 has a T1 of 27^0C which is having high level of HPMC (0.4%).The results presented in Table 1. This indicates that the mucoadhesive polymer, HPMC 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 decrease with increasing concentration of HPMC from 0.2%-1.0%w/v.

Table 1: Formulations and evaluation parameters of formulations

Formulation code	HPMC : Poloxamer(w/w)	Gelation Temperature (T1 ^{0}C)	Gel melting temperature(T2 ^{0}C)
F1	0.2: 19	31	69
F2	0.4: 19	27	62
F3	0.6: 19	24	54
F4	0.8: 19	22	50
F5	1.0: 19	21	48

Values shown in the table mean percent of three batches (n= 3)

pH of The Formulation:
The normal physiological pH of the nasal mucosa ranges from 4.5-6.5. But the nasal mucosa has the capability to tolerate pH between 3-10. pH of all the formulations was found to be between 6.9-7.5 i.e., within the range, which nasal mucosa can tolerate [23] and the results are presented in Table 2.

Drug Content:
The percentage drug content of all the formulations was found to be in the range of 97.05-99.23% (Presented in Table 2).

Table 2: Evaluation parameters of formulations

Formulation code	*pH(mean± S.D.)*	*Drug Content (mean± S.D.)*	Mucoadhesive strength (Dynes/cm2)
F1	7.1 ± 0.12	98.56 ± 0.56	2936 ± 0.72
F2	6.9 ± 0.2	99.23 ± 0.43	3838 ± 0.93
F3	7.3 ± 0.1	97.12 ± 1.02	4102 ± 0.52
F4	7.1 ± 0.13	97.05 ± 1.05	4345 ± 0.32
F5	7.5 ± 0.15	98.03 ± 0.98	4512 ± 0.36

Values shown in the table mean percent of three batches (n= 3)

Mucoadhesive Strength:
Mucoadhesive strength was determined by measuring the force required to detach the formulation from mucosal surface i.e., detachment stress. Results reveal that 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, permits intimate contact with nasal tissue, interpenetration of bioadhesive HPMC chains with mucin molecules leading to entanglement and formation of weak chemical bonds between entangled chains. Due to stronger mucoadhesive force, it can prevent the gelled solution coming out of the nose. But higher ratio of HPMC responsible for excessive bioadhesive force and the gel can damage the nasal mucosal membrane.

Viscosity:
Viscosity measurement of the formulations at various temperatures (20-40 ^{0} C), shows that there was increase in viscosity with increase in the temperature. Fig 3 shows viscosity profiles of formulations at 37^0C also reporting that mucoadhesive polymer HPMC had a viscosity enhancing effect.

***In vitro* drug release:**
The release profile of BHC from all the formulations, reveals that as the level of HPMC is increasing, the drug release is decreasing. This is due to the increasing viscosity. The retarding effect of mucoadhesive polymer, HPMC could be attributed to their ability to increase the overall product viscosity as well as their ability to distort or squeeze the extra micellar aqueous channels of PLX micelle through which the drug diffuses thereby, delaying the

release process. From the result it clearly shows that only HPMC is affecting drug release. Formulation F1, F2, F3,F4 and F5 shows the 84.12, 85.32, 80.18, 78.56 and 75.32% drug release at 5h respectively. This decrease in drug release might be due to higher level of HPMC in these formulations.

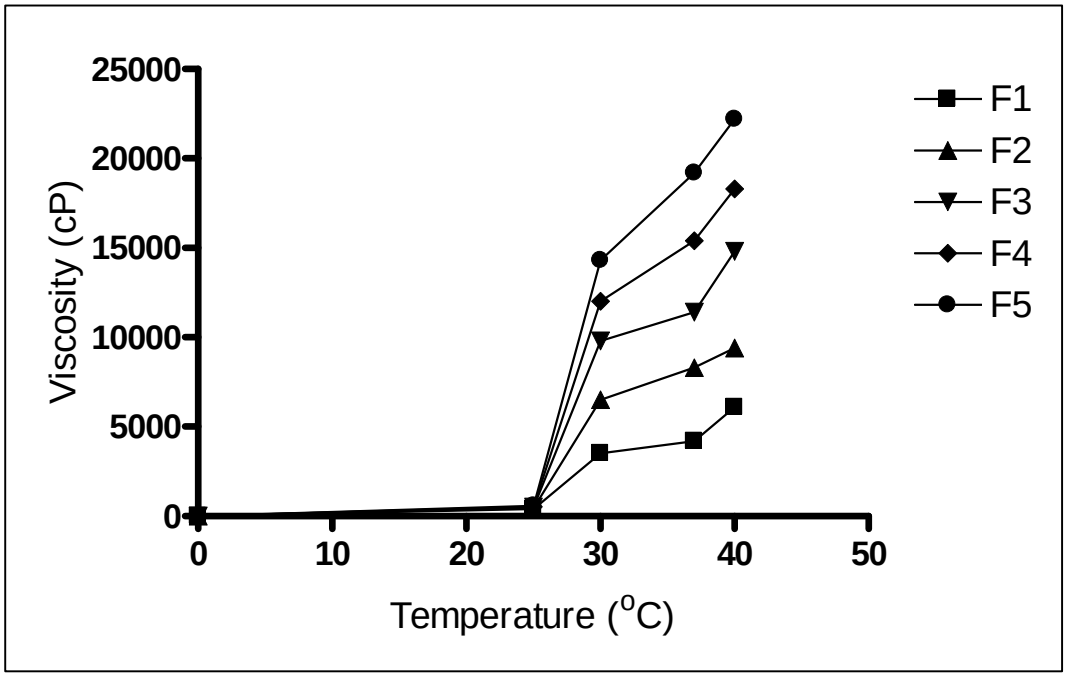

Fig 3: Viscosity measurement of formulations at various temperatures

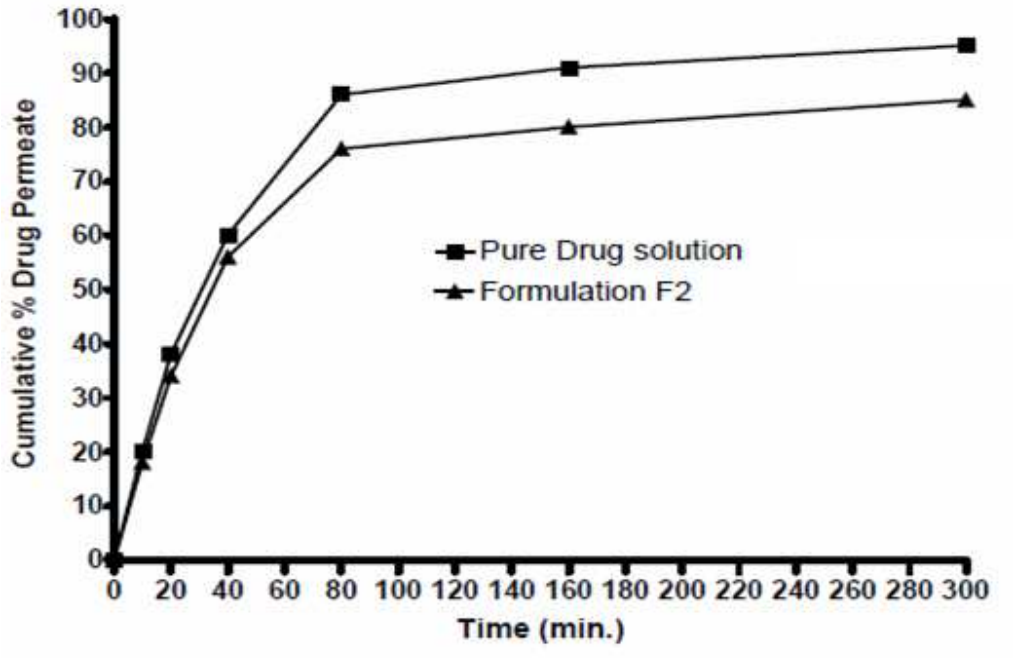

Fig 4 *: In vitro* permeation of pure drug solution and formulation F2.

***In vitro* Permetion Study:**

In vitro permeation of aqueous drug solution and formulation F2 were observed. Formulation F2 exhibited good drug release profile with favourable gelation and rheological properties. Hence, the formulation F2 was chosen as a optimized formulation, to see the permeation of drug through nasal mucosa. It was observed that permeation of drug

from aqueous solution was 95.12% where as the optimized formulation F2 shows release of 85.03 % at the end of 5 h as shown in Fig 4. The release of BHC from the gel formulation was found lower as compared to the aqueous solution, may be due to the inverse relationship between viscosity and drug release. Viscosity of the formulation influences release of BHC from gel formulation. This shows that when viscosity is increased, the release of the incorporated drug can be prolonged.

Differential Scanning Calorimetry (DSC) studies

DSC shows that crystalline BHC melts at 255˚ C, showing a sharp endothermic peak. Incorporation of BHC into HPMC and PLX did not drastically influence the thermal properties (physical mixtures). The result of DSC analysis reported that most of drug was in amorphous state, and crystalline BHC represented only a diminutive quantity of whole drug incorporated indicating absence of any drug to polymer interaction shown in Fig 5.

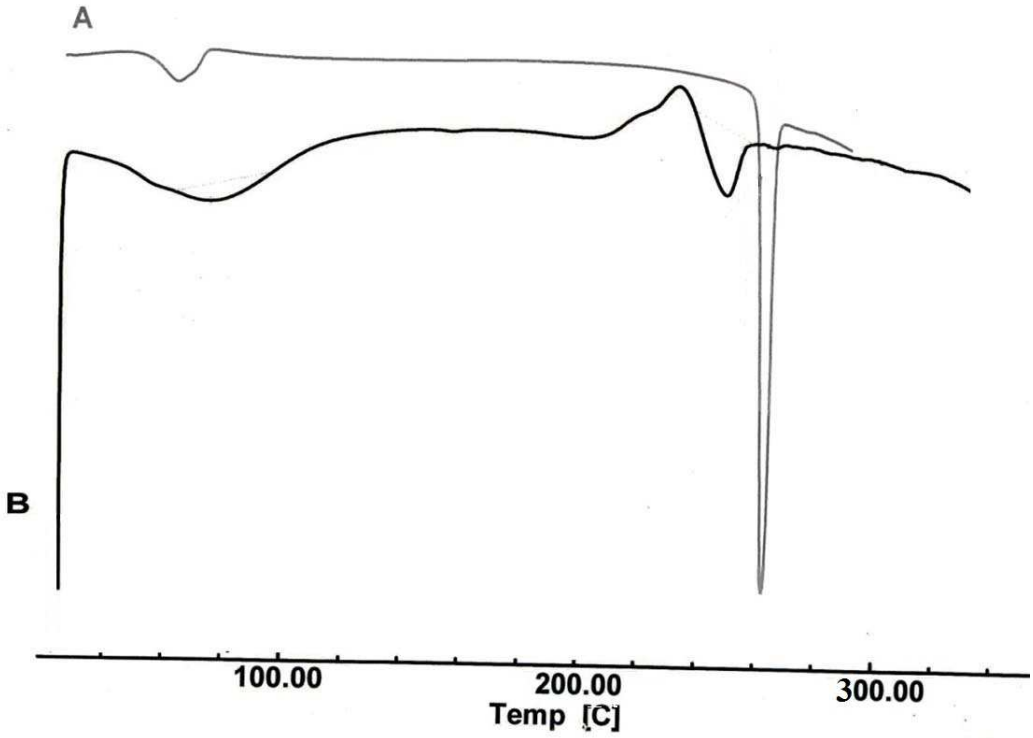

Fig 5: DSC thermpgram of pure Bromhexine hydrochloride (A) and Physical mixture (B).

CONCLUSION

In the present study, Pluronic F 127 gel with HPMC is formulated for nasal delivery of mucolytic and expectorant BHC, which would enhance nasal residence time due to its increased viscosity and mucoadhesive strength.BHC has the characteristic of presystemic metabolism of upto 80%. So, the formulation as an intra nasal gel could attribute to escaping first pass metabolism due to its administration as an *in situ* gel. Optimized formulation F2 was found out to be (composed of 1%W/V BHC , 19% W/V PLX, 0.4%W/V HPMC) better with respect to its rheological properties, gelation time, gelation temperature, pH, mucoadhesive strength, *in vitro* drug release and *in vitro* permeation studies when compared to other formulations. These results demonstrate the potential is of Pluronic F 127 and HPMC for the fabrication of controlled delivery devices for many partially water soluble or hydrophilic drugs.

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