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Nasal (*In-situ*) Gel (Phenylepherine HCl) for Allergic Rhinitis Congestion treatment: Development and Characterization

Pankaj Verma[1], Neha Prashar[1], Vikash Kumar[1], Hema Chaudhary[1]
1.P. D. Memorial College of Pharmacy, Bahadurgarh, India

ABSTRACT

The aim of the proposed work was to develop stable mucoadhesive *in situ* (Phenylepherine HCL) thermo-reversible nasal gel by cold technique and evaluated its efficacy against congestion due to allergic rhinitis. By means of hit and trial methodology screened formulations were prepare *via* numerous thermo-sensitive hydro-Gel (Pluronic F-127, Poloxamer 407) and muco-adhesives (Carbopol 934-P, Polyvinylpyrrolidone K-30, Hydroxy propyl methyl cellulose (HPMC), Sodium alginate and methylcellulose) and characterized. Out of 15 formulations; T1, T5, T7, T12 & T14 formulation compositions were selected on the basis of drug released (*in-vitro* or *ex-vivo* release found approx. up-to 97.8%) behavior, gelation and mucoadhesive strength. Resulted, drug transformed (crystalline to amorphous) form in gel state was indicated better drug absorption due to its improved physicochemical parameters. Additionally, optimized formulation (T1) histopathology and stability studied has been demonstrated none nasal mucosa damage and stable at storage conditions according to the ICH guidelines respectively. Therefore, the developed optimized thermo-reversible in-situ nasal (T1) gel was showed tremendous local safety action which can be considered to use for allergic nasal congestion and explore, further.

Keywords: Phenylephrine HCL, Mucoadhesive; Allergic Rhinitis; Decongestant; Permeation enhancers

*Corresponding Author Email: vermapankaj1614@gmail.com
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INTRODUCTION

In ancient time, nasal drug delivery was used for the systemic administration of psychotherapeutic compounds and other similar substances. But in modern pharmaceutics, nose had been considered as a route of choice for local effect rather than systemic effects. Nasal route is used for delivery of drugs used for maintenance of therapy of nasal allergy, nasal congestion, sinusitis and nasal infection[2]. Nasal congestion is the prominent symptoms in allergic rhinitis. Allergic rhinitis results from allergens such as dust, animal dander or pollen entering into nose. Because of the efficacy of topical treatment in rhinitis, topical medication are more preferred over oral systemic medication, because topical medication places a much higher concentration of decongestant on the nasal mucosa than oral decongestant. The nasal mucosa has been considered as a potential route of administration to achieve faster and higher level of drug absorption. This is due to large surface area, high blood flow, porous endothelial membrane, avoidance of first–pass metabolism and easy accessibility[1]. Mucociliary clearance, membrane permeability and environmental pH are some nasal physiological factors which affect drug absorption. The major limitation is the mechanical loss of the dosage form into the other parts of the respiratory tract like lungs due to high mucociliary clearance which results in low absorption of the drug at the site of absorption due to short residence time. In order to increase the residence time of the drug in the nasal cavity, bioadhesive formulation is used[4]. To overcome this limitation, mucoadhesion technique or mucoadhesive polymers are added in the formulation. Mucoadhesion can be defined as the state in which two components are held together for extended periods of time. In mucoadhesion, polymer which may be natural or synthetic, make intimate contact with biological membrane. After the establishment of contact ,the mucoadhesive polymer penetrate into the tissue surface. These polymers have best polarity that permits sufficient wetting by the mucus and best fluidity that allows interpenetration of polymer inside the mucus membrane.

Small and large hydrophilic drugs may be poorly permeable across nasal epithelium and may show an insufficient absorption. It is possible to greatly improve their absorption if they are administered in combination with absorption enhancers which induce reversible modifications on the structure of epithelial barrier. In intranasal drug delivery, mostly absorption enhancers used are surfactants, bile salts, fatty acids and polymeric enhancers. The mechanism of action of permeation enhancers is not well known but generally they change the permeability of epithelial cell layer by modifying phospholipidic bilayer, increases membrane fluidity or opening tight junctions between epithelial cells, hence increasing para cellular transport[4]. The types of dosage forms which are used to

deliver formulations into nose are important in determining nasal absorption profiles of drugs. Choice of certain dosage form generally depends on the drug being developed, indication being pursued and patient's compliance. Patient's compliance is prime factor in designing the formulations therefore thermos-reversible polymer are added, which undergo sol to gel transition at physiological temperature of the body and hence helps in ease of installation of formulation and accurate dosing. Thermo-reversible mucoadhesive gel is a relatively new formulation compared with powder dosage or liquid formulations[3].

The aim of this study was to formulate such a dosage form for patient suffering from nasal congestion due to allergic rhinitis using mucoadhesive polymer offering excellent adhesion to mucosal membrane of nose thereby offering longer action of the drug. Thermoreversible polymer(PF-127) was used which undergo conversion from sol to gel state at physiological temperature of the body i.e. ~32^0 C and therefore helps in ease in administration of the dosage form and hence improves patient compliance.

MATERIALS AND METHOD

Phenylephrine HCl was procured from Pharmaceutical Pvt. Ltd & other raw materials and reagents used were of standard analytical grade. The thermo reversible *in-situ* nasal gel formulation was prepared by cold method[6] (plain and drug loaded PF-127 gels were prepared with sufficient quantity of distilled water was stirred while for plain PF-127 gels, without drug was kept overnight at 4^0C in refrigerator until clear solution was obtained and finally volume was adjusted.

Optimization of plain and drug loaded PF-127 gel was done by varying the concentration of PF-127 and evaluating them for gelation temperature. Batch containing optimized concentration ofPF-127 was further used for investigation to study the effect of mucoadhesive polymers on gelation temperature and mucoadhesive strength. Three different concentrations of five mucoadhesive polymers were screened. Carbopol 934P, Hydroxyl Propyl Methyl Cellulose (HPMC), PVP K-30, Sodium alginate and Methyl Cellulose were added in the concentration 0.5, 1.0 and 1.5% respectively and were tried as a mucoadhesive polymer using logical hit and trial method[5, 7, 8].

Phenylepherine HCl, mucoadhesive polymer and benzalkonium chloride were dissolved in distilled water by agitation at room temperature. This solution was cooled to 4^0C and PF-127 was added slowly with agitation. The resulting dispersion was kept overnight at 4^0C until clear transparent solution was formed. Final volume was adjusted with cold distilled water. Evaluation of final volume was done for clarity, pH, gelation temperature, mucoadhesive strength, gel

strength, viscosity, drug content, diffusion through goat nasal mucosa, and histopathological evaluation of mucosa.

Characterization

Gelation temperature[9]:

The gelation study was done on magnetic stirrer with hot plate (Multi-Tech instrument Pvt. Ltd., India). Each formulation i.e. T1 to T15 were heated at a constant rate while stirring. The gelation point was determined when the magnetic bar stopped moving due to gelation. Each preparation was tested thrice to control the repeatability of measurement.

pH study[10]:

pH of all formulations was determined using digital pH meter (MultiTech instrument co.(p) Ltd., India) which was previously calibrated using buffers of pH 4 and pH 7 before measurement.

Appearance[10]:

All formulations were inspected visually for clarity in sol and gel form under black and white background.

Gel Strength[10]:

It is expressed in terms of time(in seconds) required by 35g weight for penetration of 5cm distance through 50 gm gel formulation. The test was performed using' Gel Strength apparatus' modified at laboratory level[22]. A sample of (50 g) was placed in a 100 ml-graduated measuring cylinder and gelled in a thermostatically controlled water bath at 37^0C.The weight (35 gm) was then placed onto the gel and strength measured in time (seconds unit required moving 35gm weight 5cm down through the gel is an indication for the viscosity of the nasal gel at physiological temperature).

Mucoadhesive strength [10, 11]:

The modified balance technique using two -glass vials and goat nasal mucosa was used. Nasal mucosa, obtained from local slaughter house, was carefully removed from the nasal cavity of goat and fixed with mucosal side out onto each glass vial using a rubber band. The vials with nasal mucosa were stored at 37^0C for 5 min in saline phosphate buffer pH 6.4 (IP); on vial with a section of mucosa was connected to the balance in inverted position while the other vial was placed on a height adjustable pan. Fixed amount (1gm) of sample of each formulation was placed onto the nasal mucosa of lower vial and height of it was adjusted so that mucosal surfaces of both vials came in contact. Contact time of two minutes was given to ensure intimate contact between tissues and the sample. Then weights were increased in the weighing pan until the two mucosal tissues got detached from each other.

Viscosity Studies[12, 13]:

The rheological studies were carried out using Brookfield viscometer (Brookfield Engineering Laboratories, Inc Middleboro, MA).The gel formulation under study was placed in sample holder and the suitable spindle selected was lowered perpendicular into the sample. The spindle was rotated at constant optimum speed to determinations of formulation viscosity at 4^0C as well as 34^0 C.

In Vitro Release Studies[10]:

In vitro release study of the formulation was performed in two chamber diffusion cells through dialysis membrane-70 with molecular weight cut off 1200-1400 KDa. To prepare artificial membrane, pieces of dialysis membrane were hydrated in saline phosphate buffer solution (pH 6.4) IP for 30 min before mounting on diffusion cell. In situ gels of PF-127 loaded with drug were placed in the donor compartment.10 ml of saline phosphate buffer solution (pH6.4) IP was placed in the receptor compartment. The temperature of the receiver compartment maintained at 37^0C±1.0^0C during experiment and content of the receptor compartment was stirred with a magnetic stirrer. The position of the donor compartment was adjusted such 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 hour interval and replaced with same amount of fresh medium to maintain sink condition. Samples were stored in refrigerator until analyzed using UV spectro-photometer at 273.5 nm for drug. In vitro release was carried out for 5 hrs.

In Vitro Permeation Studies[7, 15]:

A fresh nasal mucosa carefully removed from the nasal cavity of goat obtained from the local slaughterhouse was used for *ex -vivo* studies. Nasal mucosa separated from sub layer bony tissues and lipids were removed from blood with 1ml of chloroform-methanol (2:1 v/v) for 60 min in order to extract the lipids from mucosa. Before permeation studies mucosa was hydrated with saline phosphate buffer solution (pH 6.4) IP at 4^0C and the permeation studies were carried out. Nasal mucosa was mounted onto donor chamber with serosal surface towards receptor chamber. The diffusion medium used was saline phosphate buffer (pH 6.4) IP. The receptor chamber was filled with PBS and continuously agitated with magnetic stirrer. The cell was maintained at 37±2^0C and 0.25% w/v of test formulation was placed on dorsal mucosal surface and position of donor chamber was adjusted such that serosal surface just touches the diffusion medium. Then 1 ml of sample was withdrawn from the receptor chamber at predetermined time interval from 30 min to 480 min and replaced with fresh medium (PBS) maintained at 37^0C. The sample withdrawn

was diluted to 10 ml with PBS and drug content was carried out by UV method. The diffusion studies were carried out in triplicate.

Mathematical Kinetic Assessment for Drug Release Mechanism [16]:

The release kinetics is an integral part for development of a dosage form. If the kinetics of drug release is known, *in vivo in vitro* (IVIVC) correlation can also be established. Mathematical approach is the scientific methods to optimize and evaluate the error in terms of deviation in the release profiles of formulated products during the formulation development stage. The release kinetics was applied for the optimized formulation. The drug release kinetics of the formulation was studied by fitting the data in different kinetic models and assessing the correlation coefficient.

***In vitro* drug release data were fitted to kinetic models:**

The criteria for selecting the most appropriate model are lowest sum of square of residuals (SSR) and highest R^2 value. Lowest sum of square of residuals (SSR) indicate the minimum variance between the predicted and observed dissolution data and find R^2 value which indicates linearity of dissolution data.

Table 1: Diffusion value with overall solute diffusion mechanism

Diffusion Exponent Value (n)	Overall Solute Diffusion Mechanism
0.45	Fickian diffusion
0.45<n<0.89	Non fickian diffusion or anomalous transport
n=0.89	Case II transport

Stability Studies:

Optimized *in- situ* gel was stored at 4^0 C ± 0.5^0temperature for 3 months. Then appearance, pH, % drug content and viscosity were investigated. For estimation of drug content; a**s** per ICH guidelines, optimized formulation were subjected to to 4^0C ± 0.5^0 for 3 months at the end of 30, 60, and 90 days, samples were withdrawn diluted with saline phosphate buffer IP (pH 6.4) and analyzed with UV Spectrophotometer[16].

RESULTS AND DISCUSSION

Using logical hit and trial approach, preliminary trials were conducted to optimize the range of excipients going to be used in thermo reversible *in-situ* nasal gel formulation. Gelation temperature for PF-127 based gels were observed for the concentration range of 16-20% (G1 to G3), and it was found that the gelation temperature of PF-127 based gels decreases with increase in concentration of PF-127.When drug was added into gels, it was found that gelation temperature of formulation (G4 to G6) increased significantly for each concentration of gelling agent shown in Table 2; however, the pattern was similar. The decrease in the gelation temperature with increase in PF-127

concentration may be due to the higher number and volume occupied by micelles at low temperature. As the concentration of PF-127 increases, the gel structure becomes more closely packed with the arrangement in the lattice pattern and at low temperature, gelling occurs rapidly. Incorporation of drug into in- situ nasal gels increases gelation temperature. This may be due to water soluble nature of PE which may cause modification of the process of micellar association of PF-127gels thereby increasing their gelation temperature[10, 18]. From results (Table 2), it was found that only 18% of PF-127 gel with drug (G5) showed ability to form gel in the range of 29^0 to 32^0 C. Hence, 18% (w/v) concentration of PF-127 was used for further studies.

Table 2: Results of optimization of concentration of PF-127

Formulation	G1	G2	G3	G4	G5	G
PE-HCl(%w/v)	xxx**	**	**	10	10	10
PF-127(%w/v)	16	18	20	16	18	20
Gelation Temperature(^{0}C)	34±0.31	28.6±0.25	23.7±0.30	36.5±0.50	32.3±0.49	23.7±0.30

Values are expressed as mean ± SD; n=3 and ** signifies blank.

For the optimization of mucoadhesive polymer, different mucoadhesive polymers namely carbopol 934, PVP K 30, HPMC, sodium alginate and methylcellulose were used in the concentration of 0.5, 1.0 and 1.5. Concentration of drug was kept constant i.e. 10 % (w/v) and of preservative i.e. Benzalkonium chloride to be 0.01% (w/v) in all formulations (T1-T15). Mucoadhesive polymer was optimized on the basis of effect of mucoadhesive strength and effect of addition of mucoadhesive polymer on gelation temperature. Formulation batches from T1 to T15 with different mucoadhesive polymers in different concentration were evaluated for mucoadhesive strength and gelation temperature Table 3.

Mucoadhesive polymer make intimate contact with biological membrane, after the establishment of contact, the mucoadhesive polymer penetrate into the tissue surface. Hence mucoadhesive polymer helps to prevent mucociliary clearance of the drug and prolong the drug's contact time with the mucus membrane[19, 20]. With increasing concentration of mucoadhesive polymer, mucoadhesive strength increases. The mechanism of mucoadhesion may be due to hydrogen bonding between gel formulation and mucosal membrane[22]. Mucoadhesive strength is dependent on strength of polymer bonding with membranes which vary from polymer to polymer and on mechanism of mucoadhesion, hence, difference in mucoadhesive strength of different polymers was observed.

Evaluation of 5 Selected Formulations:

As concentrations of mucoadhesive polymer increased, there was significant decrease in gelation temperature and increase in mucoadhesive strength. It was found that formulation T1, T5, T7, T12 and T14 showed optimum results in terms of mucoadhesive strength and gelation temperature. Hence, concentration of mucoadhesive polymer was selected for further study. All prepared sets of formulations were found to be clear when observed under black and white background. Lysozyme is found in nasal secretions, which is responsible for destroying certain microbes at acidic pH and at alkaline pH, lysozyme is inactive and nasal tissue is prone to microbial infection. It is therefore advisable to keep pH of the formulation in the physiological range. pH of all formulations was found to be in the range of 6.0 to 6.4 which is considered as nasal physiological pH range.

The temperature at which phase transition from solution to gel occur is noted as the gelation temperature. The gelation temperature of in-situ gelling formulations T1, T5, T7, T12 and T14 was determined by visual inspection method. From the results obtained from gelation study it was seen that all the five formulations batches showed gel formulation at physiological temperature. The gelation time is defined as the time taken for transition of liquid phase to a gel. In the present study, gelation time was found to be within 2 min.

Table 3: **Formulation table for batches T1 to T15 with gelation temperature and mucoadhesive strength.**

Composition(%w /v)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**	**T12**	**T13**	**T14**	**T15**
Phenylephrine	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PF-127	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
Carbopol 934	0.5	1	1.5	*	*	*	*	*	*	*	*	*	*	*	*
PVP K-30	*	*	*	0.5	1	1.5	*	*	*	*	*	*	*	*	*
HPMC	*	*	*	*	*	*	0.5	1	1.5	*	*	*	*	*	*
Sodium alginate	*	*	*	*	*	*	*	*	*	0.5	1	1.5	*	*	*
Methylcellulose	*	*	*	*	*	*	*	*	*	*	*	*	0.5	1	1.5
BKC	0.01	0.01	0.01	0.01	0.01	0.1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Distilled water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Gelation Temperature (C)	31± 0.23	29.2± 0.31	28.3 ± 0.31	34.8 ±0.2 0	30.2 ± 0.30	28.8 ±0.6	30.2± 0.31	28.63 ±0.26	28.23 ±0.42	33.2 ±0.3 1	34.0± 0.382	32.8± 0.58	33.1 ±0.6	31.0 ±0.1 4	28.5± 0.224
Mucoadhesive strength (dyne/cm2)	1,160 ± 5.20	2 496 ± 5.81	2820 ±5.2 0	865 ± 5.36	998 ± 3.48	106 0 ±5.4	1,061 ± 3.48	1,780 ± 8.66	2,505 ± 8.66	590 ± 4.42	865 ± 5.34	1,160 ± 5.20	620 ±5.7	991 ± 4.33	1,125 ± 3.43

Values are expressed as mean ± SD ; *signifies blank; q.s.= Quantity sufficient;

From viscosity studies, it was concluded that all formulations were in liquid state at room temperature and converted into gel at nasal physiological temperature. From the drug release profile, it was concluded that initial rate of release from each formulation was very rapid, this may be due to incomplete gel formation in the earlier time period, release became slow in latter period after complete gel formation.

Table 4: Viscosity of formulations at 4^0C and 32^o C.

Formulation Batch no.	T1	T5	T7	T12	T14
Clarity	++	++	+++	+++	++
pH	6.2 ± 0.04	6.0 ± 0.05	6.0 ± 0.03	6.4 ± 0.04	6.3 ± 0.02
Gel Strength (sec)	28.7±1.53	37.3±0.58	32.3±0.58	46.3 ±1.53	42.3 ± 1.15
Viscosity at					
4^oC	50.1±3.06	55.5±0.99	34.2±2.34	43.4± 1.04	67.8 ± 2.56
32^o C	150.1±2.13	166.2±1.08	115.1±2.62	115 ± 2.11	180 ± 2.33

Values are expressed as mean ± SD; n=3. ++ is clear and +++ is very clear (glassy)

***In-vitro* drug permeability study:**

The study was performed by using dialysis membrane. *In-vitro* study was performed by Franz diffusion cell using dialysis membrane and saline phosphate buffer (pH 6.4 IP) was used as a diffusion medium. The drug release (depicted in Table 5 & 6) profile was obtained by plotting percent drug release against time.

Table 5: *In-vitro* drug released data in saline phosphate buffer IP (pH 6.4)

Drug Release (in percentages) at numerous time (in minute) intervals								
S. No.	**Codes**	**0**	**30**	**60**	**120**	**180**	**240**	**300**
1.	T1	0	33.1±1.23	38.4±2.34	47.7±2.22	68.7± 0.91	85.9± 1.20	97.8± 1.32
2.	T5	0	29.4± 1.33	34.3±1.67	44.1±1.43	65.1± 1.22	80.5±1.46	95.3±1.11
3.	T7	0	31.2±1.42	36.4±1.93	44.3±1.23	67.1± 2.12	83.2± 1.23	96.7± 1.33
4.	T12	0	29.0± 3.40	35.0± 1.34	43.1± 1.08	64.6± 1.54	80.2± 1.2	94.5± 1.43
5.	T14	0	27.2±1.23	32.4± 2.12	41.8± 2.02	62.8± 1.42	78.1± 3.21	92.1± 2.59

The results showed that the formed gels had ability to retain Phenylepherine HCl for the duration of 300 min. T1formulation showed higher release of 97.8 % than others. Hence T1 is selected for further evaluation and is the optimized formulation.

Table 6: **Optimized formulation (T1) *in-vitro* release data (pH 6.4)**

Time (min)	SQ RT Time (min)	Log time (min)	% CDR	Log % CDR Retained (T1)	Log % CDR (T1)
30	0.71	1.48	33.10	1.82	1.52
60	1.00	1.78	38.40	1.79	1.58
120	1.41	2.08	47.70	1.72	1.68
180	1.73	2.25	68.74	1.49	1.84
240	2.00	2.38	85.88	1.15	1.93
300	2.24	2.48	97.80	0.34	1.99

***In-vitro* release kinetics:**

To establish the order and mechanism of drug release, *in-vitro* release data of the optimized formulation (T1) were fitted to four different kinetic models, namely Zero order model, First order model, Higuchi model and Korsmeyer Peppas model. The model for best fit was predicted from the value of R^2. During evaluation regression is higher for Zero order kinetics (Table 7) which indicated that the drug release kinetics is Zero order kinetics whereas the n value obtained for Korsmeyer indicates non fickian diffusion mechanism of drug release.

Table 7: **Value of Regression (R^2) obtained from different kinetic models**

Formulation Code	Zero Order	First Order	Higuchi Model	Korsmeyer Peppas model
Formulation T1	0.98	0.84	0.95	0.93

Evaluation of optimized formulation (T1):

The physical state of drug incorporated or dispersed in polymeric matrix was determined by XRD analysis of drug loaded in sol and gel state. The characteristic sharp diffraction peaks obtained for pure drug were not detected in diffractogram obtained for optimized formulation in sol and gel state, indicating complete dispersion of drug in amorphous state in hydrophilic core of semi crystalline polymer matrices. Therefore, it could be concluded that the nature of drug changes from crystalline to amorphous in sol and gel state. It therefore increases absorption rate.

***Ex vivo* Permeation Studies:**

The permeation study of optimized formulation (T1) with drug solution (control) was carried out by using Franz diffusion cell apparatus in which goat's nasal mucosa was used as a diffusion membrane and PBS at pH 6.4 was used as a diffusion medium. Drug release profile was obtained by plotting percent drug release against time, and results of permeation study are given in Table 8. Ex-vivo permeation study was carried out with T1 formulation and drug solution (control) in saline phosphate buffer IP (pH 6.4). From drug release profiles, it was concluded that the initial release rate from T1 formulation was rapid, this may be due to incomplete gel formation at earlier time period but release became slow in latter time after complete gel formation. Ex-vivo permeation study indicated sustained release of T1 as compared to control due to muco-adhesive polymer used in formulation. From drug release profiles, it was concluded that T1 formulation initial release rate was rapid; may be due to incomplete gel formation at earlier time period but release became slow in latter time after complete gel formation. *Ex-vivo* permeation study indicated sustained release of T1 compare to control which could be due to mucoadhesive polymer used in formulation. The release profiles exhibited an inflection point, which indicated gel formation on diffusion membrane

in donor compartment of diffusion cell. During gel formation, formulation was converted into gel phase and hence drug release became slow.

Table 8: Ex-vivo drug permeation data

S.No.	Formulation code	% Drug Release					
		0min	30 min	60 min	120 min	180 min	240 min
1	T1	0	31.4±0.90	36.5±1.12	45.3±0.89	65.1±0.71	80.5±0.82
2	Control	0	39.3±1.29	44.3±2.3	53.5±0.88	74.3±3.3	90.8±0.93

Table 9: Ex-vivo absorption data of Optimized Formulation T1 and Control (Drug solution)

Time (min)	Sq Rt Time (min)	Log Time (min)	% CDR T1	Log % CDR Retained T1	Log % CDR T1
30	0.70	1.48	31.40	1.84	1.5
60	1	1.78	36.54	1.80	1.57
120	1.41	2.08	45.30	1.74	1.66
240	2	2.38	80.54	1.29	1.90

Table 10: Value of R^2 obtained from different kinetic models

Formulation Code	Zero Order	First Order	Higuchi Model	Korsmeyer Peppas model
Formulation T1	0.98	0.93	0.93	0.92

Results depicted in Table 10 showed that the drug follows Zero order kinetic and n value obtained from the *Peppas* model describes the mechanism of drug release as non-fickian diffusion.

Histopathological Evaluation of Nasal Mucosa

Goat nasal mucosal membrane was used for the ex-vivo permeation study. The optimized formulation T1 showed good permeation through mucosal membrane. The histopathology study of nasal mucosa showed no damage to the tissue. Histopathological study revealed that formulation T1 was not causing necrosis to nasal mucosa as it does not cause damage or detachment to the epithelial cells and mucus secreting glands. Hence T1 formulation was found to be safe as it was harmless to nasal mucosa.

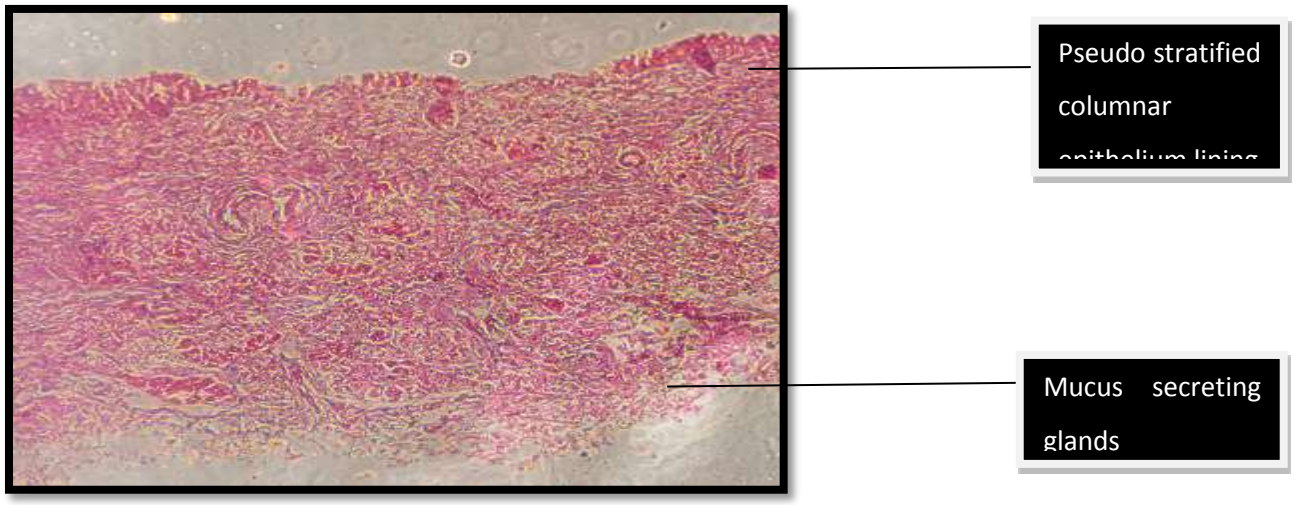

Figure 5: Microscopy of normal nasal mucosa (Control)

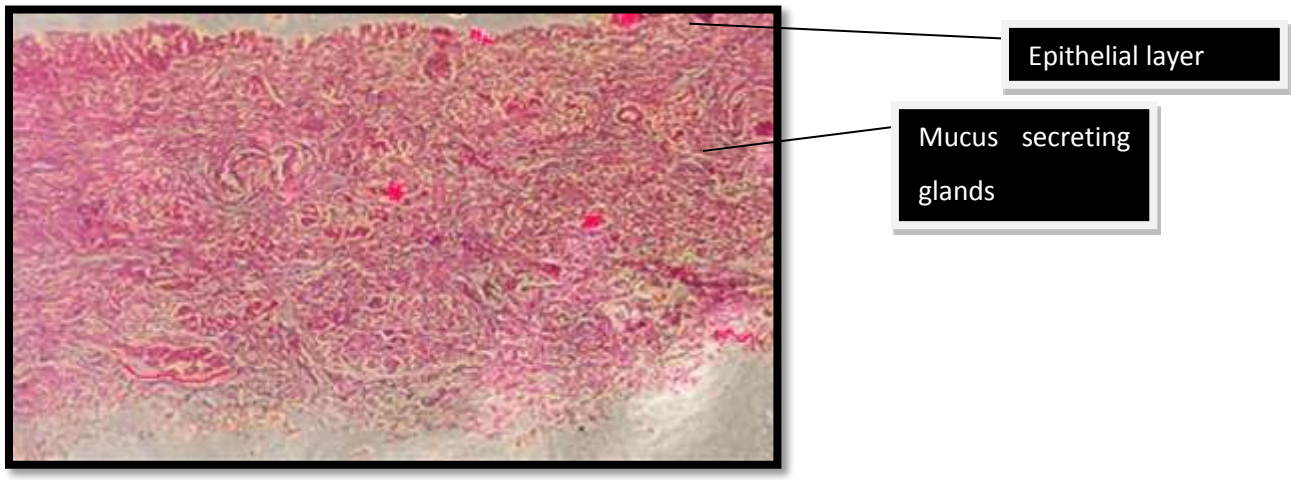

Figure 5B: Microscopy of nasal mucosa on exposure to formulation T1

Stability as per ICH guidelines: The stability studies were carried out as per ICH guidelines Q1A (R2) for optimized formulation (T1) at normal & accelerated storage conditions i.e. 4^0C ± 0.5%, 25 ± 2^0C respectively, for 3 months. The physical changes appeared in formulation exposed to different storage conditions were observed at certain time interval during 3 months of stability studies and results are summarized in Table 11.

Table 11: **Physical and Chemical Stability Study of Optimized Formulation (T1).**

Time (days)	Temperature(^{0}C)	Appearance	Viscosity	pH	Drug content (%)
0	4 ± 0.5	Clear	54.5 ± 2.34	6.4± 0.05	97.2 ±1.22
30	4 ± 0.5	Clear	54.5 ± 2.34	6.4± 0.04	96.2 ± 1.30
60	4 ± 0.5	Clear	54.5 ± 2.33	6.4± 0.05	95.2 ± 1.23
90	4 ± 0.5	Clear	54.5 ± 3.22	6.4± 0.04	95. 1 ± 1.20

There is no significant physical change observed in *in-situ* nasal gel formulation during stability study at the above mentioned storage conditions of temperature indicating good physical stability of formulations in terms of appearance and viscosity. The change in chemical stability parameters i.e. drug content and pH were also found to be non-significant. The shelf-life of optimized formulation gel (T1) was determined by sigma-plot is 435 days.

CONCLUSION

The in-situ nasal gel of Phenylepherine HCl was successfully prepared by cold method for local action in nasal congestion due to allergic rhinitis. The F_{opt} was found to be T1.T1 formulation has shown good *in-vitro* and *ex-vivo* release. The drug was transformed from crystalline to amorphous form in gel state, indicating better and improved physicochemical properties of drug for absorption. Overall, the results of this study show tremendous promise of thermo-reversible *in-situ* nasal gel for local action. The histopathology of optimized formulation showed no damage to nasal mucosa hence the formulation is safe for use. This strategy can potentially be extremely useful.

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REFERENCE

1. Chein YW, Su KS, Chang SF. Nasal systemic delivery. 2nd ed., .New York: Dekker; 1989: 26-27.
2. Akhtar A, Prajapati SK, Singh D, Kumar B, Kausar S. Enhanced bioavailability of drugs via intranasal drug delivery system. Int Res J Pharm; 2012,3(7). 68-74.
3. Singh S, Kanupriya, kumar SLH .Thermoreversible Mucoadhesive Gels As Promising System For Nasal Drug Delivery: A Review, International Journal of Natural Product Science 2012, Spl Issue 1:238.
4. Pires A, Fortuna A, Alves G, Falcão A. Intranasal Drug Delivery: How, Why and What for? Journal of pharmacy and pharmaceutical science 2009; 12(3): 288-11.
5. Mandar JB, Amelia MA. Thermo reversible Nasal *In Situ* Gel Of Venlafaxine Hydrochloride: Formulation, Characterization, and Pharmacodynamic Evaluation. AAPS PharmSciTech.Mar 2013;14(1): 101-10.
6. Schmolka IR. Poloxamers in the pharmaceutical industry. In:Tarch PJ, editor. Polymers for controlled drug delivery. Boca Raton, FL: CRC Press; 1991: 189-14.

7. Uttarwar S. Formulation and Development of *In Situ* Gelling System for Nasal Administration for an Antiemetic Drug Ondansetron Hydrochloride by Using Pluronics 127P and Pluronics 68. IJRBPS 2012; 3(3):1103-16.
8. Parmar VJ. Formulation and development of Thermoreversible Mucoadhesive Intranasal *In-Situ* hydrogel by using a combination of polymers (poloxamers 407 and 188). Bulletin of Pharmaceutical Research 2012; 2(3): 167-74.
9. Pathan BI , Chudiwal V, Farooqui I, Shingare P. Formulation design and evaluation of nasal *in situ* gel as a novel vehicle for Azelastine hydrochloride. Int Journal of Drug Delivery 2013; 284-90.
10. Mandar JB, Amelia MA. Thermo-reversible Nasal *In-Situ* Gel of Venlafaxine Hydrochloride : Formulation, Characterization, and Pharmacodynamic Evaluation. AAPS Pharm Sci Tech 2013; 14 (1): 101-10.
11. Sharwaree H. Performed Development of Mucoadhesive Nasal In-Situ gel to Sustain the Release of Amitriptyline Hcl. IJBPR, 2012; 3(8): 980-89.
12. Ricci EJ, Bentley MVLB, Farah M, Bretas RES, Marchetti JM. Rheological Characterization of Poloxamer 407 lidocaine hydrochloride gels. Eur J P harm Sci 2002; 17: 161-167.
13. Shinde JV, Mali KK, Dias RJ, Havaldar VD, Mahajan NS. Insitu Mucoadhesive Nasal Gels of Metoclopramide Hydrochloride: Preformulation and Formulation Studies. J Pharm Research. 2008; 1(1) : 88-96.
14. Karasulu HY, Sanal ZE, Sozer S, Guneri T. Permeation Studies of Indomethacin from Different Emulsions For Nasal Delivery and Their Possible Anti –Inflammatory Effects. AAPS Pharm Sci Tech 2006; 7 (3): E80-86.
15. Dash S, Murthy PN, Nath L. Kinetic modeling on drug release from controlled drug delivery systems. Acta Poloniae Pharmaceutica –Drug Research 2010; 67(3): 217-23.
16. Guidance for Industry Q1 A(R2) Stability Testing of New Drug Substances and Products. [Internet] Centre for Drug Evaluation and Research; Fishers Lane; Rockville. Available from http://www.fda.gov/download/Drugs/GuidanceCompliance RegulatoryInformation/Guidance/ucm073369.pdf (access on month date , year)
17. Chien YW, Su KSE, Chang SF. Nasal Systemic Drug Delivery. New York: Marcel Dekker; 1999: 39-78.
18. Devillers G. Exploring a pharmaceutical Market Niche &Trends: Nasal Spray Drug Deliver. Drug development and delivery 2003; 3(3): 1-4.

19. Wen MM. Olfactory targeting through intranasal delivery of biopharmaceutical drugs to the brain-Current Development. Discovery Medicine 2011; 11: 497-03.
20. AbdElhady SS, Mortada N, Awad GAS, Zaki NM, Taha RA. Development of *in situ* gelling and mucoadhesive mebevarine hydrochloride solution for rectal administration. Saudi Pharm J 2003; 11: 159-71.
21. Dumortier G, Grossland JL, Agnely F, Chaumeil JC. A Review of Poloxamer 407 Pharmaceutical and Pharmacological Characteristics. Pharm Res 2006; 23: 2709-27. doi:10.1007/s11095-006-9104-4.
22. Yong CS, Choi JS, Quan QZ, Rhee JD, Kim CK. Effect of Sodium Chloride on Temperature, Gel Strength and Bioadhesive Force of Poloxamer Gels Containing Diclofenac Sodium. Int. J. Pharm 2001; 226: 195-05.