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RESEARCH ARTICLE

Evaluation of *Blatta orientalis* (*Q*) nasal gel formulation in milk aspiration induced eosinophilia

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Abstract

Background: The purpose of the present study was to develop intranasal delivery systems of the homeopathic anti-asthmatic remedy *Blatta orientalis* mother tincture (*Q*) using thermoreversible polymer Pluronic F127 (PF127) and mucoadhesive polymer Carbopol 934P (C934P).

Methods: Formulations were modulated so as to have a gelation temperature below 34°C to ensure gelation at physiological temperature after intranasal administration. Its gelation temperature, mucoadhesive strength, viscosity and gel strength were studied. *B. orientalis* (*Q*) nasal gel was tested with recurrent milk aspiration to determine whether it produces changes in eosinophilia in a murine model of asthma.

Result: The gelation temperatures of the formulations and mucoadhesive strength, determined using sheep nasal mucosal membrane, increased by the addition of increasing concentrations of Carbopol. The results of milk aspiration induced eosinophilia, *B. orientalis* (*Q*) nasal gel significantly ($P < 0.001$), decreased eosinophil cell count as compared with toxicant by using in absolute eosinophilia count method. Finally, histopathological examination did not detect any damage during in vivo studies.

Conclusion: The PF127 gel formulation of *B. orientalis* (*Q*) with in situ gelling and mucoadhesive properties with increased permeation rate is promising for prolonging nasal residence time and thereby nasal absorption.

Keywords: Blatta orientalis (Q)*; Carbopol; asthma; mucoadhesive; nasal gel; Pluronic F127R*

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Introduction

Allergic asthma is one of the common diseases that affect mankind with diverse manifestations. The prevalence of allergy and asthma has risen in recent years despite an improvement in the general health of the population.[1] Various economic studies have identified the causes for increase in the prevalence of upper and lower respiratory tract allergic diseases. Some of the postulated reasons are increasing environmental pollution[2] and increased predisposition of individuals producing excessive IgE antibody through a major change in the gene pool, eosinophil cells, and mast cell degranulation in the asthmatic disorders.[3]

Homeopathy has described several drugs from plant, insects, and animal sources for use in the treatment of bronchial asthma. Dynamizations of *Blatta orientalis* (*Q*) have been found to have a strong in vivo inhibitory effect on IgE production, eosinophil granulocytes, which play a fundamental role in the acute asthmatic patient. But *B. orientalis* (*Q*) has a very fishy smell to take by oral route. Bioavailability via nasal route is equivalent to intravenous route. So for status asthmatic condition (acute) faster absorption should be there. Nasal gel can be the better alternative from a drug delivery point of view. Hence, a formulation that would increase residence time in the nasal cavity and at the same time increase absorption of the drug would be highly beneficial in all respects.[4]

The use of bioadhesive polymers can lengthen the residence time and enhance bioavailability of drugs delivered to the nasal cavity.[5,6] Polymer gels and mucoadhesive polymers have been studied for the mucosal delivery of various compounds ranging from small molecule to macromolecular drugs. The nasal bioadhesive gels might be used to provide an enhanced bioavailability

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compared with oral delivery. Application of in-situ gelling solutions of tri-block copolymers of poly (ethylene oxide) and poly (propylene oxide) (Pluronic) exhibiting thermo-reversible properties have been proposed to lower the viscosity of the nasal formulations below the body temperature.[7] By modulating the gelation temperature of different PF127 solutions, liquid bases for nasal use can be formulated that form a gel in the nasal cavity at body temperature with suitable gel strength resulting in enhancement of the residence time in the nasal cavity. Gelation of concentrated solutions of Pluronics is a well-documented phenomenon related to the appearance of tightly packed cubic liquid crystalline micellar phases.[8] The high solubilizing capacity and nontoxic properties of PF127 make it suitable for nasal drug delivery. The objective of this study was to develop an intranasal delivery system of *B. orientalis* (*Q*) using thermo-reversible polymer PF127 and mucoadhesive polymer Carbopol 934P (C934P), with various concentrations, which would enhance nasal residence time and absorption of drug across nasal-mucosal membrane. The pharmacodynamic activity of nasal gel was studied by animal model.

Experimental

Materials

Chemicals

Mother tincture of *B. orientalis* (*Q*) was purchased from Rani medical store, Dhule. PF127 was procured from (Sigma chemicals). C934P was supplied from (Lubrisol chemicals) as a gift sample. All other materials and solvents obtained from commercial sources were of analytical grade. Ultra-pure water was used throughout the studies.

Animals

Inbred Wistar rats (150–200 g) and guinea pigs (300–400 g) of either sex were housed in standard conditions of temperature (22 ± 2°C), with relative humidity (60 ± 5%) and 12 h light/dark cycles. They were fed with standard pellet diet and water *ad libitum*. The study received approval from the Institutional Animal Ethics Committee (IAEC) of R.C. Patel Shirpur, India, and was in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on Animals (CPCSEA); registration number RCPCOP/IAEC/2007-08/25.

Methods

Preparation of mucoadhesive polymer-based thermo-reversible nasal formulations

PF127 and *B. orientalis* (*Q*) were solubilized in distilled water containing 1% propylene glycol. The PF127 vehicles used throughout this study were composed of 18% wt/vol of PF127. The concentration of PF127 was selected so as to obtain thermoreversible gel at minimum possible concentration. PF127, 18% wt/vol, was found to be the lowest concentration (when formulated in addition of *B. orientalis* [*Q*]) that exhibited thermoreversible property below 34°C (temperature of the nasal cavity).[4,6] Hence, 18% wt/vol of PF127 was selected for further studies. The liquid was left at 4°C until a clear solution was obtained. Thermoreversible gels were prepared using cold method. Bioadhesive anionic polymer C934P was slowly added to the solution with continuous agitation. C934P was added in concentration range of PLC1 = 0.1% wt/vol, PLC2 = 0.3% wt/vol, PLC3 = 0.5% wt/vol to PF127 solution. PLB was prepared the blank gel for comparison[4,6] (see Table 1).

Measurement of gelation temperature by visual inspection [4]

Gelation temperature, defined as the temperature at which the liquid phase makes a transition to gel, was determined as described previously.[9] In brief, a 10-mL transparent vial containing a magnetic bar and each formulation were placed in a water bath. The vial was heated at a constant rate while stirring. The gelation temperature was measured when the magnetic bar stopped moving due to gelation. Each preparation was tested thrice to control the repeatability of the measurement.

Viscosity measurement method

Viscosities of formulations before and after gelation were measured with a Brookfield Viscometer (Brookfield DV-E) spindle no. 3 at 100 rpm shear rate.[4]

Evaluation of the mucoadhesive strength[9]

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from nasal mucosal tissue.[10] The bioadhesive force of PLB, PLC1, PLC2 and PLC3 gels were determined by using measuring device in (Figure 1) In brief, a section of tissue was cut from the nasal cavity of sheep obtained from the local slaughterhouse instantly secured with mucosal side out onto each glass vial (C) using a rubber band and an aluminum cap. The vials

Table 1. PF127, C934 and *B. orientalis* (*Q*) gel formulation.

Formulation	PF127 (% wt/vol)	C934P (% wt/vol)	Drug (% vol/vol)
PLB	18	-	-
PLC1	18	0.1	*B. orientalis* (*Q*) 1
PLC2	18	0.3	
PLC3	18	0.5	

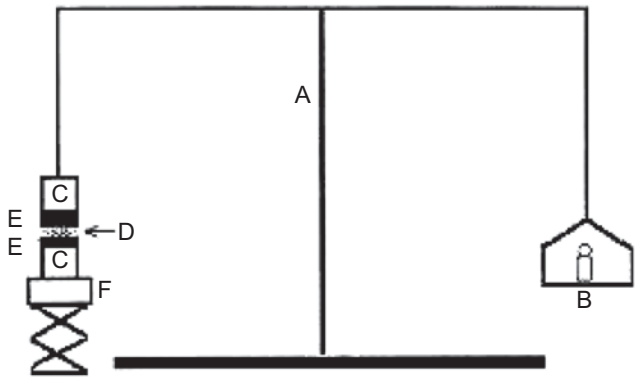


Figure 1. Mucoadhesive strength-measuring device.

with the nasal tissues were stored at 36.5°C for 10 min. Next, one vial with a section of tissue (E) was connected to the balance (A) and the other vial was placed on a height-adjustable pan (F). PLB, PLC1, PLC2 and PLC3 gels (D) were added onto the nasal tissue on the other vial. Then, the height of the vial was adjusted so that the PLB, PLC1, PLC2 and PLC3 gels could be placed between the mucosal tissues of both vials. The weights (B) kept raised until two vials were attached. Bioadhesive force, the detachment stress (dyne/cm^2), was determined from the minimal weights that detached two vials. The nasal tissue pieces were changed for each measurement.[6,9]

Detachment Stress (dyne/cm2) = m × g/A

Where **m** is the weight added to the balance in grams; **g** is the acceleration due to gravity taken as 980 cm/s^2; and **A** is the area of tissue exposed. Measurements were repeated three times for each of the gel preparations, but before each measurement a fresh smooth gel surface was created.

Effect of contact time of gel on mucoadhesive strength

Effect of varying contact time (1, 3 and 5 min) was investigated for some of the gel preparations to optimize initial contact time. In brief, formulations were allowed to be in contact with mucosa for carrying contact times (1, 3 and 5 min), and the bioadhesive force was determined as discussed above. Contact time that resulted in maximum bioadhesive strength was selected as optimum contact time required for adequate adhesion.[4]

Measurement of gel strength

PLC1, PLC2 and PLC3 gels (50 g) was put in a 100 mL-graduated cylinder and gelled in a thermostat at 36.5°C. The apparatus for measuring gel strength (weight: 35 g) was then placed onto the gels (Figure 2). The gel strength, which means the viscosity of PLC1, PLC2 and PLC3 gels at physiological temperature, was determined

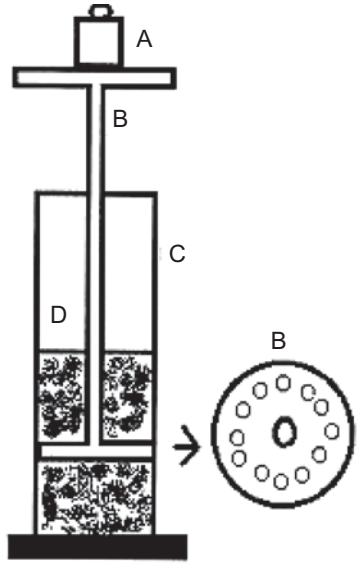


Figure 2. Gel strength-measuring device.

by the time (s) the apparatus took to sink 5 cm down through the gels. In cases that it took more than 300 s to drop the apparatus into the gel, various weights were placed on the top of the apparatus, and the gel strength was described by the minimal weights that pushed the apparatus 5 cm down through the gel.[9]

Pharmacological screening of nasal gel of B. orientalis (Q)

Recurrent milk aspiration produces changes in eosinophilia in a murine model of asthma [11,12]

Purpose and rational. Recurrent aspiration of foreign material into the respiratory tract is a common problem in children and patients with predisposing anatomic and functional disorders such as tracheoesophageal fistula, gastric esophageal reflexes GER, and neuromuscular disorders.[12,13]. An association between milk aspiration and the development of airway dysfunction has been suggested by clinical and laboratory studies in recent years.[14–17]. In this respect, limited information is available on the role played by immune cells in the development of altered airway function seen in response to milk aspiration.

The only one stable nasal gel PLC2, showing no inters and intra chemical changes in ingredients were selected. For determining the dynamic property of *B. orientalis* (*Q*) gel by using milk aspiration induced eosinophilia model was performed.

Procedure

Cow's milk was used as the aspiration and sensitization medium. After sedation with ketamine

(30 mg/kg), or anesthetic ether, 50 μL of milk was placed into the nose of quietly breathing mice while the mouth was closed. This was the same modified technique described by Colasurdo (1998)[18] and Gelfand (1997).[17] Swiss mice were divided into four groups six in each. Group I (normal) was normal saline treated (non-sensitized), while the sensitized mice of Group II were treated with 10 μL of PLC2 nasal gel of *B. orientalis* (*Q*) intranasaly and Group III was treated with *B. orientalis* (*Q*) 0.6 mL p.o. Milk was allowed to aspirate into the lungs with strong respiratory effort as evidenced by the coughing produced with inoculation of either material. The procedure did not lead to the awakening of the mice, and the coughing resolved within 30–60 s after completion of the nasal injection of milk or saline. This protocol was used for single aspiration. The latter was accomplished by instilling 50 μL of milk daily for 10 days. In Group II, mice were aspirated 10 μL PLC2 (*B. orientalis* [*Q*] gel formulation), half an hour after milk aspiration on alternative days until 10 days, while Group II mice received 0.06 mL *B. orientalis* (*Q*) on alternative days orally after milk aspiration until 10 days. Blood was collected from retro-orbital plexus under light ether anesthesia. A total of 24 h after of the last aspiration event eosinophil count was taken by absolute eosinophil count method using eosin dye. Histopathological evaluations of nasal mucosa of mice were carried out at Sai Pathology Laboratory, Aurangabad (Maharashtra) India.

Statistical analysis

The results of various studies were expressed as mean ± SEM and analyzed statistically using ANOVA with Tukey-Kramer multiple comparison and Bonferroni's multiple comparison post-hoc test to find out the level of significance using GraphPad Prism-5. The minimum level of significance was fixed at $P < 0.05$.

Results

Mucoadhesive polymer-based thermo-reversible nasal formulations

Measurement of gelation temperature by visual inspection

Data shown in Table 2 of the gelation temperature was obtained using two different methods like visual inspection and viscosity method. Based on visual identification, the in situ gel has remained liquid for six months (Table 2). It was noted that the addition of increasing concentrations of C934P from 0.1–0.5% further increased the gelation temperature from 27.67–35.33°C. The formulation containing the concentration of C934P 0.5% was found to have very high solution-gel transition temperature, and so was difficult to administer into the nostril.

Table 2. Gelling temperature determined rheologically and by visual inspection (n = 3).

Formulations	Gel strength (sec) at 36.5°C	Viscosity (cP)		Solution-gel transition temperature (°C) by visual inspection*
		Solution	Gel	
PLB	111.6	91	1050	27.67 ± 0.881
PLC1	137	114	1800	32.09 ± 0.577
PLC2	153.3	138	4970	34.04 ± 1.202
PLC3	179.1	227	5843	35.33 ± 1.202

*Values are mean ± SEM.

Viscosity measurement method

A marked increase in viscosity was observed in all the formulations of Carbopol. The viscosity of formulations both in sol and gel state; was found to be proportionate with the increasing Carbopol polymer concentration (Table 2).

Effect of contact time of gel on mucoadhesive strength

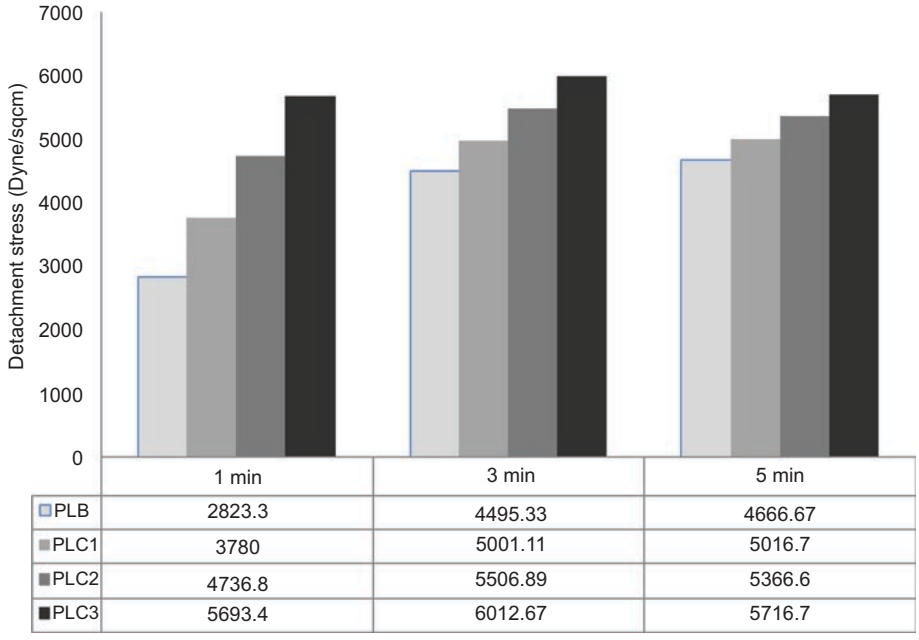
Three minutes of contact time was found to give optimum mucoadhesive strength. Further increase in contact time did not have significant effect on mucoadhesive strength, whereas decreased contact time resulted in less mucoadhesive strength resulting from insufficient time for entanglement of polymer chains with mucin (Figure 3). Assessment of the mucoadhesive strength in terms of detachment stress showed that the PF127 preparations possessed adhesive properties that increased with the addition of C934P concentration. Mucoadhesive strength was increased from formulations PLC1, PLC2, and PLC3 with 0.1%, 0.3%, and 0.5% C934P concentration, respectively, as compared with PLB control.

Gel strength

At 36.5°C, the gel strength of PLB was found to be less as compared PLC1, PLC2, and PlC3. Concentration of C934P was directly proportional to gel strength (Table 2).

Recurrent milk aspiration produces changes in lung eosinophilia in a murine model of asthma

On the 10th day eosinophil cell count of mice was taken by absolute eosinophilia method. Total eosinophil cell count on 10th day of normal mice was found to be 66.6 ± 11.4 cells/cumm (Figure 4), while toxicant group mice (only milk-treated) showed significant raise in eosinophil cell count up to 333 ± 26.88 cells/cumm. Test group PLC2 (*B. orientalis* [*Q*] nasal gel treatment) showed significant ($P < 0.001$) reduction in eosinophil cell count 107.3 ± 17.5 cells/cumm as compared to toxicant. PLC2 nasal gel of *B. orientalis* (*Q*) showed 67.78% anti-eosinophilic activity at very low concentration (10 μL), while the *B. orientalis* (*Q*) orally-treated group showed 58.88% anti-eosinophilic activity. The *B. orientalis* (*Q*) showed



	1 min	3 min	5 min
PLB	2823.3	4495.33	4666.67
PLC1	3780	5001.11	5016.7
PLC2	4736.8	5506.89	5366.6
PLC3	5693.4	6012.67	5716.7

Figure 3. Time-dependent mucoadhesive strength study of PLB, PLC1, PLC2, PLC3, gels.

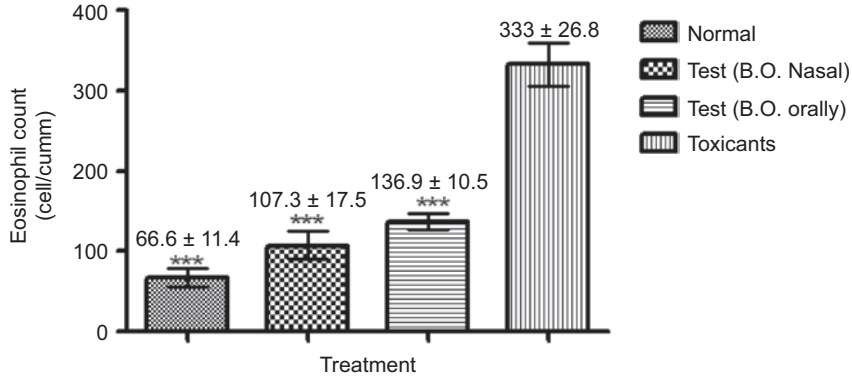


Figure 4. Effect of *B. orientalis* (*Q*) on aspiration of milk induced eosinophilia.

more prominent anti-eosinophilic activity in PLC2 gel formulation, than orally treated. This difference may be due to the drug bioavailability of nasal gel is more than the oral route (Figure 4).

Mucosal toxicity study

The microscopic observations indicated that the optimized formulation has no significant adverse effect on the microscopic structure of mucosa. As shown in Figure 5, neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after treatment of PLC-2 (Figure 5b) as compared with nasal mucosa of toxicant group mice (Figure 5c). The epithelium layer was damaged and alterations in basal membrane and superficial part of submucosa as compared with normal mice mucosa (Figure 5a).

Discussion

Gelation temperature is an important parameter for in situ nasal gel for administration purpose. If the gelation temperature of a thermoreversible formulation is lower than 25°C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering. If the gelation temperature is higher than 37°C, a liquid dosage form still exists at the body temperature, resulting in the nasal clearance of the administered drugs at an early stage. As the temperature of the nasal cavity is 34°C,[19] this study aimed at preparing the liquid formulations of PF127 that may gel around 34°C.[4]

The gelation of PF127 vehicles is known to result from the change in micellar number with temperature. With

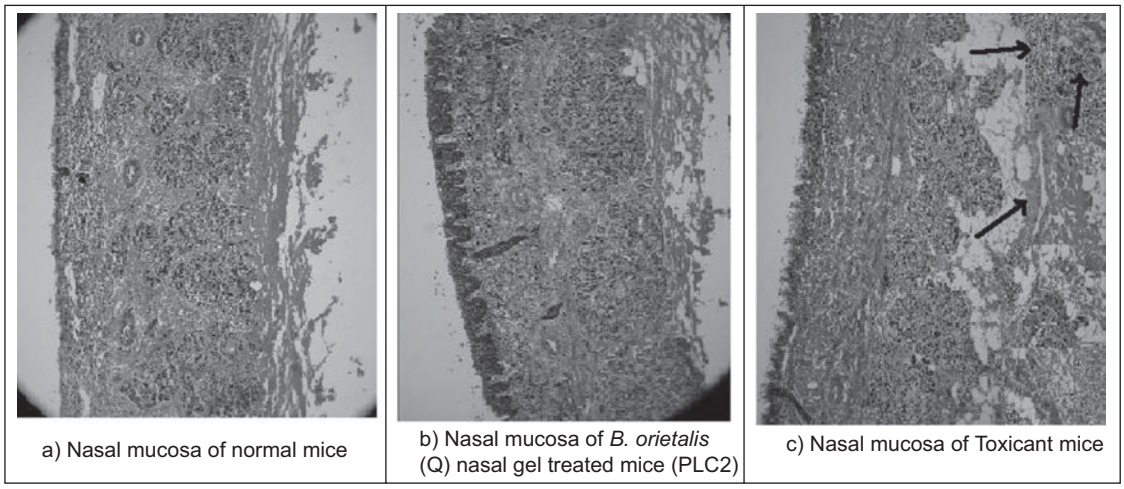


Figure 5. Histopathological evaluations of sections of mice nasal mucosal membrane.

increasing temperature, the number of micelles formed increases as a consequence of the negative coefficient of solubility of block copolymer micelles. Eventually the micelles become so tightly packed that the solution becomes immobile and gel is formed. Recently, Majithiya et al. (2006)[4] suggested a mechanism of gelation based on micelles packing and entanglements. Also, conformational changes in the orientation of the methyl groups in the side chains of poly (oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon. The gelation temperature-lowering effect of mucoadhesive polymers might be caused in part by the increased viscosity after dissolution of mucoadhesive polymers. Formulations containing C934P concentration higher than 0.5% were found to have very high viscosity, and so were difficult to administer into the nostril. Regardless of the concentration of mucoadhesive polymers, all the PLC1, PLC2, PLC3 formulations gelled at the temperature ranging from 27.67–35.33°C. These temperatures seem to be proper for in situ gelling of the various vehicles at the nasal cavity, minimizing the loss of administered drug caused by clearance from the site of application.[4]

Mucoadhesive strength is the very important parameter as far as nasal gel concern. Earlier work with Carbopol polymers has clearly indicated that it is the availability of carboxyl groups that determines bioadhesion; Carbopol has a very high percentage (58–68%) of carboxylic groups that gradually undergo hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane, resulting in formation of a strengthened network between polymer and mucus membrane. Thus, Carbopol, having high density of available hydrogen bonding groups, would be able to interact more strongly with mucin glycoproteins. In addition, Carbopol may also adopt more favorable macromolecular conformation with increased accessibility of its functional groups for hydrogen bonding. It is speculated that the higher mucoadhesive strength of the delivery system may lead to the prolonged retention and increased absorption across mucosal tissues.[4] Mucoadhesive strength was increased from formulations PLC1, PLC2, and PLC3 with 0.1%, 0.3%, and 0.5% C934P concentration, respectively, as compared with PLB control. This means that concentration of C934P is directly proportional to mucoadhesive strength.

In the development of in situ nasal gel, the gel strength is important in finding the condition, which allows the easy administration of the gel and no leakage from the [9]. Thus, the ranges of gel strength suitable for nasal gel system were investigated by inserting PF127 gels into the nose of a mouse and observing any leakage after administration. From this observation two thresholds in gel strength; the upper and the lower limit was final out. Above the upper threshold of gel strength, it was difficult to insert the PLC gels. Under the lower limits, the PLC gels leaked out from the nose. The result indicates that concentration of C934P was directly proportional to gel strength.

PLC2 gel was selected for the screening, because it has good mucoadhesion property, and it was stable for three months without gelling at room temperature and it convert into gel at 34°C as compared with PLC1 and PLC3. PLC3 remained liquid after three months but after administration into the nostrils of mice, it leaked out due to its high gelling point. PLC1 was converted into gel in a few days at room temperature. PLC2 remain liquid after three months and after administration into the nostrils of mice converted to gel and stuck there. This was due to various concentrations of C934P, so PLC2 was selected for pharmacological screening. The availability and non-degradability was analyzed pharmacodynamicaly.

PLC2 was analyzed by 'recurrent milk aspiration produces changes in lung eosinophilia in a murine model of asthma'. The *B. orientalis* (*Q*) containing PLC2 gel showed more prominent anti-eosinophilic activity than orally treated. This may have been because the drug bioavailability of nasal gel is more than the oral route. PLC2 gel has good mucoadhesive strength and leads to more contact time for absorption which reflects the bioavailability of drug in the body. Absorption of *B. orientalis* (*Q*) by the nasal route was higher than the oral route. The nasal route also avoids first pass metabolism which may lead to more bioavailability to remain active in the blood. *B. orientalis* (*Q*) required only 10 μL of 1% gel to show its activity as compared to the oral route which required a 60 μL dose. This low dose of nasal gel may avoid aggravation of asthma. A histopathological study also showed no erosion or necrosis nor removal of the epithelium. Thus, gel formulations seem to be safe with respect to nasal administration. Results conclude that the administration of *B. orientalis* (*Q*) by nasal route will be more effective than the oral route.

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