Research Article

Formulation Development and Evaluation of In-Situ Nasal Gel of Lisinopril Dihydrate

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Abstract: The present study was aimed to develop a mucoadhesive In-situgel of Lisinopril Dihydrate for improved bioavailability by circumventing the hepatic first pass metabolism and patient compliance. Lisinopril dihydrate was incorporated into the blends of thermo reversible polymer pluronic F 188(PF 188) and bio adhesive polymer Carbopol 934 in the form of In-situgel by cold technique to reduce the mucociliary clearance, and thereby it will increase the contact of formulation with nasal mucosa and hence improving drug absorption. The prepared gels were characterized by pH, Drug content, Gel strength, in -vitro drug release studies, stability study etc. The pH of all the formulations were found to be within the range between 4.5-6.5 and the nasal mucosa can tolerate the above mentioned pH of the formulations. The drug content of all formulations was found to be 91.09 to 99.98%. Viscosity measurement of the formulations at temperatures 25°C & 37°C shows that there was increase in viscosity with increase in the temperature and it was found that all formulations were in liquid state at room temperature and were converted into gel at nasal physiological temperature. The optimized formulation showed a drug release of 98.83% in 8 hrs.
Keywords: Lisinopril dihydrate, In-situgel, nasal delivery, Pluronic F188.

INTRODUCTION

Nasal mucosa has also been considered as a potential administration route to achieve faster and higher level of drug absorption because it is permeable to more compounds than the gastrointestinal tract due to lack of pancreatic and gastric enzymatic activity, neutral pH of the nasal mucus and less dilution by gastrointestinal contents [1]. The nasal epithelium is a highly permeable monolayer; the sub mucosa is highly vascularised with large and fenestrated capillaries facilitating rapid absorption. Moreover, direct systemic absorption avoids hepatic first-pass metabolism [2, 3].

Lisinopril dihydrate is the long acting angiotensin-converting enzyme inhibitor class that is primarily used for treatment of hypertension, congestive heart failure, heart attack and also in preventing renal and retinal complication of diabetics. Lisinopril dihydrate is a lysine derivative of enalapril at and does not require hydrolysis to exert pharmacological activity. Lisinopril dihydrate (2S)-1{[2S)-6-Amino-2-{[1S) carboxy3phenylpropyl] amino] hexanoyl] pyrrolidine-2 carboxylic acid dihydrate It is given orally, it is undergoes extensive first pass metabolism. Lisinopril dihydrate has a narrow absorption with only 25% of the drug being absorbed in the GIT .The half-life of its accumulation is 12 hours [7].

In-situgels are the novel drug delivery systems that favors the ease and convenience of administration and delivery of accurate dosage forms which are the major problems encountered by the normal semi solid dosage forms. An in situ mucoadhesive gel appears to be very attractive since they are fluid like prior to administration which makes them easy to administer as a drop allowing accurate dosing. Formulation of in situ gels depends on various physical and chemical stimuli [4-6].

Poloxamers or Pluronics are a class of thermo reversible gels that have the capacity to make, break and modify the bonds responsible for holding the network together. Their thermo reversible property make them useful as a carrier for most routes of administration including oral, topical, intranasal, vaginal, rectal, ocular and parenteral routes. Reverse thermal gelation and low toxicity have been the basis of research into the use of Pluronics as a possible drug delivery system in man [8].

MATERIALS AND METHODS
MATERIALS

Lisinopril dihydrate was obtained as a gift sample from JCPL, PVT, LTD, Jalgaons, Poloxamer-188 was obtained as a gift sample from Signet chemicals and BASF chemical company, Navi Mumbai. Propylene Glycol, Benzalkonium chloride and triethanolamine used were LR grade.

METHOD
Cold technique
Formulation and Preparation of Nasal in situ Gel

Accurately weighed quantity of the drug was dissolved in distilled Water. The solutions of Poloxamer-188 and Carbopol-934 were prepared using cold method. A certain volume of distilled water was cooled down to 4°C. Poloxamer-188 and Carbopol 934 was sprinkled over deionised cold water separately and was allowed to hydrate for 12 hours to produce a clear solution. Then both the polymer solutions were mix properly with continuous stirring. The Benzalkonium chloride was added to the above polymer dispersion. Then stored in the refrigerator. The dispersions were then stored in a refrigerator until clear solutions were obtained and polymer dispersion was slowly added to the drug solution under aseptic condition. The formulation was aseptically transferred to previously sterilized glass bottles and sealed [6].

Composition of different batches of gel formulation

Table 1: Composition of different batches of gel formulation as per 3^2 factorial designs

Formulation code / Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Lisinopril dihydrate (% w/v)	5	5	5	5	5	5	5	5	5
Poloxamer-188 (% w/v)	14	16	18	14	16	18	14	16	18
Carbopol-934 (% w/v)	0.1	0.1	0.1	0.15	0.15	0.15	0.2	0.2	0.2
Propylene Glycol (% w/v)	1	1	1	1	1	1	1	1	1
Benzalkonium Chloride (% w/v)	1	1	1	1	1	1	1	1	1
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Purified water (ml)	100	100	100	100	100	100	100	100	100

EVALUATION OF GEL
Physical parameter
pH

pH of each formulation was determined by using Digital pH meter (Digital pH meter 335). This was previously calibrated by pH 4 and pH 7. The pH values were recorded immediately after preparation [9].

Viscosity

The rheological properties of gels were determined by the Brookfield viscometer; type DV-II + PRO using spindle no LV-3 (63). Viscosities of the formulations were taken at two different temperatures i.e. at room temperature and at 37°C with varying shear rate [10].

Measurement of the gel strength

A sample of 25 mL of the gel was put in a 50 mL graduated cylinder. A weight of 14.33 g was placed on the gel surface. The gel strength, which is an indication for the ophthalmic gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel. All measurements were performed in triplicate (n=3) [11].

Mucoadhesive Strength

"Detachment Stress is the force required to detach the two surfaces of mucosa when a formulation/gel is placed in between them". The detachment stress was measured by using a modified analytical balance [12].

Force of adhesion (N) = (bio adhesive strength/1000) $\times$ 9.81
Bond strength (N/m^2) = force of adhesion (N)/surface area of disk (m^2)

Drug Content

1ml of the formulation was added in 100ml of distilled water to make 100ppm stock solution and from that 1ml was taken and adjusted with10ml distilled water to give 10ppm and at the fixed wavelength the absorbance of the formulation was carried out [11].

In-vitro Drug Release Study

In-vitro release study of the formulated In-situgel was carried out by using diffusion cell through goat nasal mucosa. Diffusion cell with inner diameter 1.4cm was used for the study. The formulation 1 ml were placed in donor compartment and Freshly prepared 100 ml simulated nasal electrolyte solution (sodium chloride 0.745gm, potassium chloride 0.129 gm, calcium chloride dehydrated 0.005gm, distilled water q.s. 100ml) in receptor compartmentnasalmucosawere mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that nasal mucosajust touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was

maintained at 37°C ± 0.5°C. 1ml of sample is withdrawn from receiver compartment after 30 min, 1, 2, 3, 4, 5, 6, 7 & 8 hrs and same volume of fresh medium is replaced. The withdrawn samples was diluted to 10ml in a volumetric flask with distilled

water and analyzed by UV spectrophotometer at specific wavelength [9].

Stability Studies
        Test conditions for stability study are shown in (Table 2).

Table 2: Test Conditions for Stability Study

Test Conditions	
Duration of study:	3 months
Temperature conditions:	Room temperature $25^0C\pm2^0C$
Relative humidity conditions:	$60\pm 5\%$
Frequency of testing the samples:	30 Days

        The formulations were evaluated mainly for their physical characteristics at the predetermined intervals of 90 Days like appearance/clarity, pH, viscosity and drug content.

RESULT AND DISCUSSION
pH
        The pH of all the formulations from F1 to F9 was found to be in the range of 6.0 to 6.4 pH values of formulations shown in Table No.3
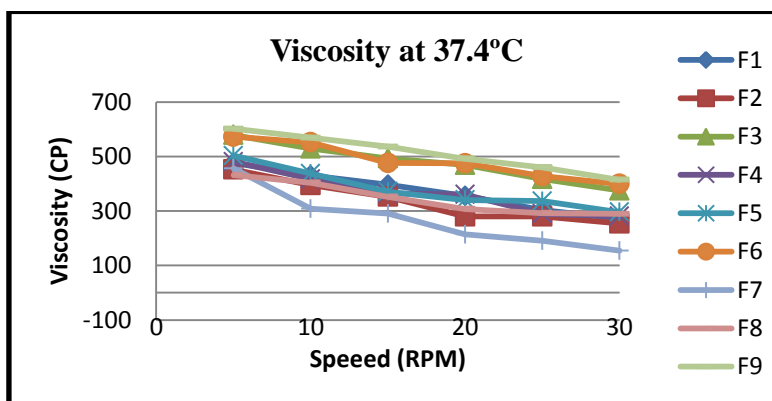
Viscosity
        The Viscosity profile of formulations at room temperature and at 37°C is shown in Figure 1 and 2respectively.

        Viscosity v/s rpm plots for all formulations shows decrease in viscosity as shear rate (rpm) was increased. Which indicate that, gel has the pseudo plastic flow? As temperature was increased the increase in viscosity was observed because of temperature sensitive polymer (poloxamer 188) was used in the formulation. Concentration of poloxamer 188 was a major factor affecting viscosity of formulations. In combination with poloxamer 188 and Carbopol 934 has shown considerable increases in viscosity when concentration of poloxamer-188 was 14% w/v to 18%w/v.
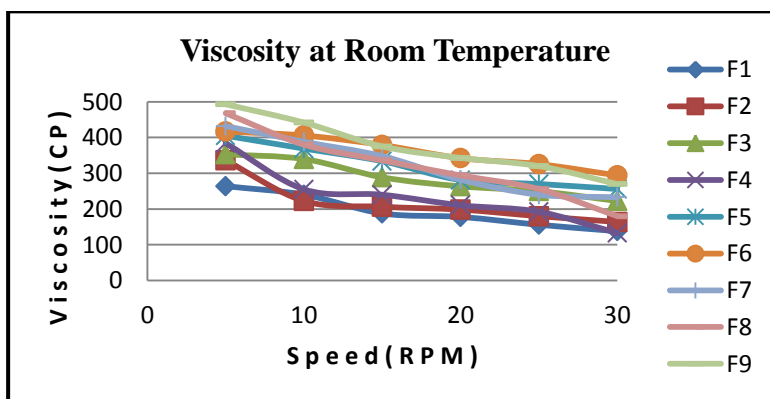
Fig 1: Viscosity profile of formulations at room temperature

Fig 2: Viscosity Profile of Formulations at 37.4°C

Measurement of the Gel Strength

The gel strength of Nasal formulation shown in Table no.3

Mucoadhesive strength

The detachment stress of formulation is shown in Table No.3. Mucoadhesive force means the force with which gels bind to nasal mucosa. Greater mucoadhesion is indicative of prolonged residence time of a gel and thus prevents its drainage from nasal cavity. The mucoadhesion force increased significantly as the concentration of mucoadhesion polymers increased. The Detachment Stress was determined for nasal gels. Results of this test indicate that the variable

Poloxamer-188 and Carbopol 934 both are having effect on mucoadhesive strength. It shows that mucoadhesive force was increased with the increasing concentration of the Poloxamer-188 and Carbopol 934.

Drug content

The Drug content of formulations is shown in Table No.3 .The optimized formulation drug content is found to be 99.98%

In-vitro drug release study

The drug release of optimized batch was found to be 98.83% shown in Table no. 3. The drug release profile of formulation shown in Fig. no.3
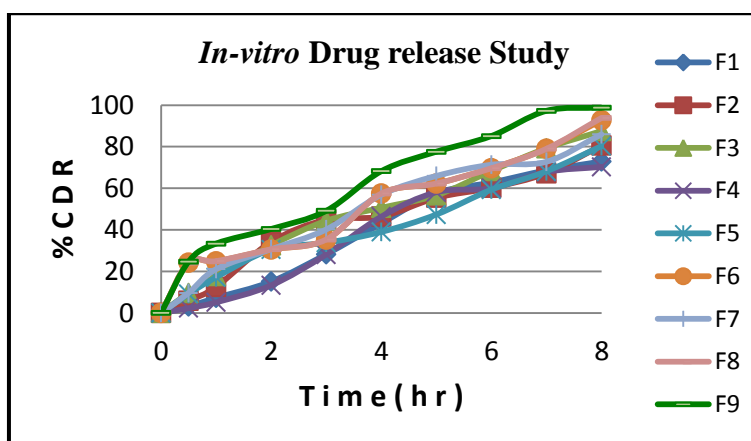

Fig 3: In-vitro drug release profile of formulation

Table 3: Physical evaluation of In-situ nasal gel

Formulation	pH (±SD)	Gel strength (sec) (±SD)	Mucoadhesive strength (Dynes/cm^2	Drug content (%) (±SD)	Cumulative drug release (%) (±SD)
F_1	6.0 ± 0.1	0.43 ± 0.02	0.0519±0.0007	93.8±1.44	70.34 ± 0.0032
F_2	6.3 ± 0.1	0.48± 0.01	0.0549±0.0001	96.87±0.50	76.54 ± 0.0001
F_3	6.23± 0.1	0.54 ± 0.017	0.0619±0.0001	94.68±0.02	87.65 ± 0.0001
F_4	6.1± 0.12	0.78 ± 0.012	0.0694±0.0007	92.71±0.02	70.31 ± 0.0001
F_5	6.34 ± 0.1	0.85 ± 0.015	0.0784±0.0007	91.09±0.12	90.54 ± 0.0001
F_6	6.2 ± 0.03	0.92 ± 0.015	0.0813±0.0001	96.39±0.24	91.45 ± 0.0001
F_7	6.1 ± 0.1	1.14 ± 0.026	0.0917±0.0007	95.39±0.12	85.95 ± 0.0001
F_8	6.3 ± 0.1	1.24 ± 0.012	0.1050±0.0007	96.66±0.12	95.09 ± 0.0013
F_9	6.4 ± 0.1	1.45± 0.02	0.1113±0.0001	99.98±0.24	98.83 ± 0.001

Optimization

A 3^2 full factorial design was selected and the 2 factors were evaluated at 3 levels, respectively. The percentage of Poloxamer 188 (X_1) and Carbopol 934 (X_2) were selected as independent variables and the dependent variable was % drug release, viscosity and Mucoadhesive strength. The data obtained were treated using Design expert version 7.0.0 software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D

response surface methodology to study the interaction of Poloxamer 188 (X_1) and Carbopol 934 (X_2) on dependent variable. ANOVA for the dependent variable % drug release (Y_1), Mucoadhesive Strength (Y_2) and Viscosity (Y_3). The values of X_1 and X_2 were found to be significant at p <0.05, hence confirmed the significant effect of both the variables on the selected responses. From this data optimum concentration of Poloxamer 188 18% w/v and Carbopol 0.2% w/v was found.
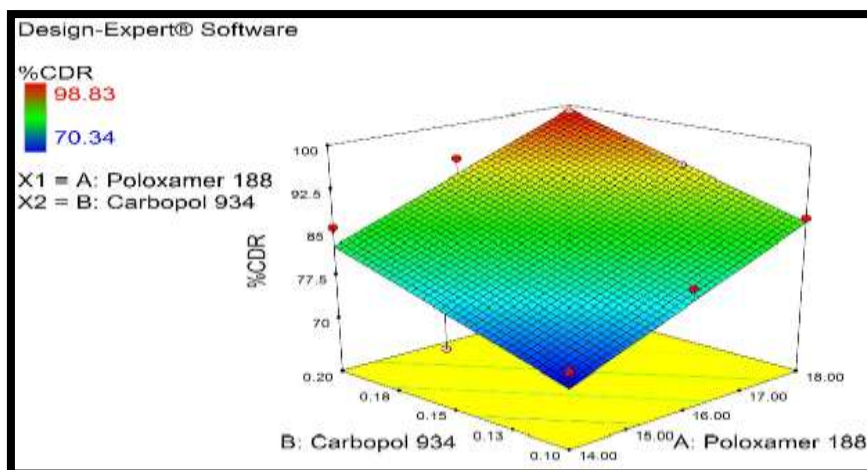
Fig.4: Surface response plot showing effect of Poloxamer 188 and Carbopol 934 on drug release
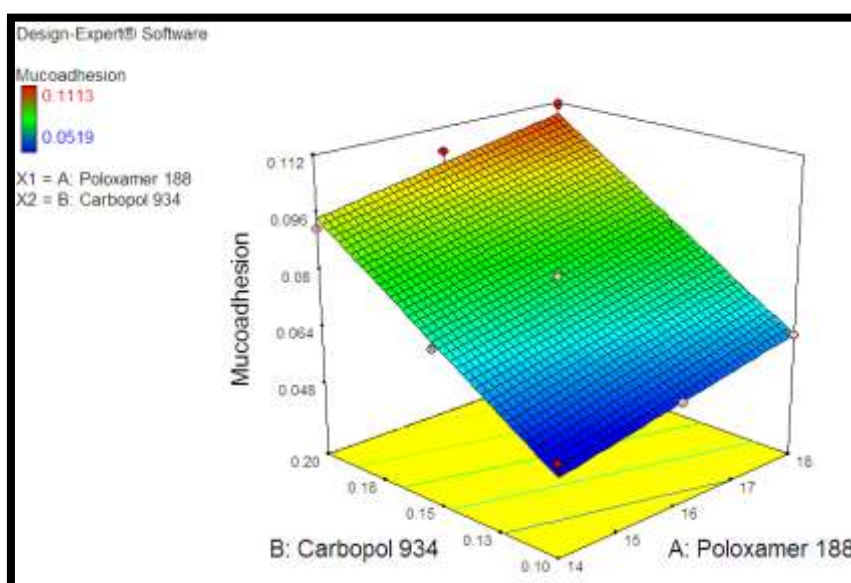

Fig 5: Surface response plot showing effect of Poloxamer 188 and Carbopol 934 on Mucoadhesive strength
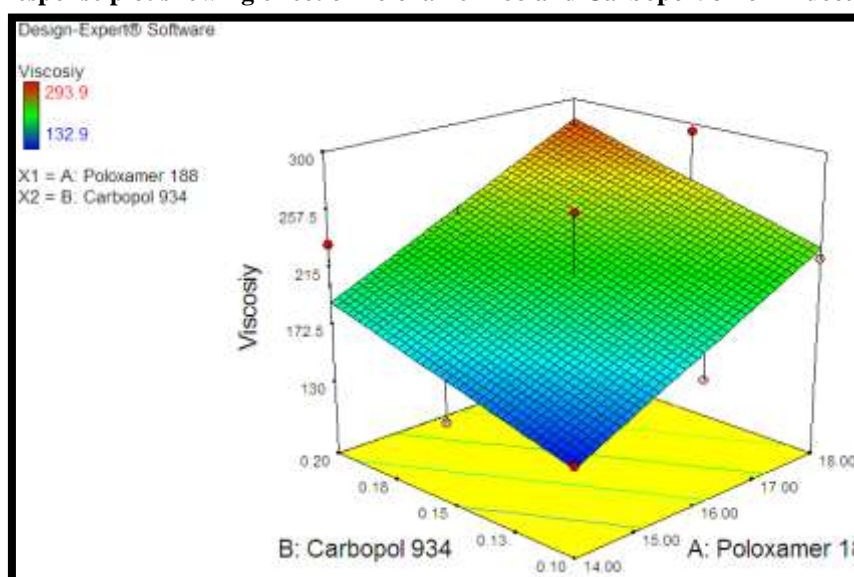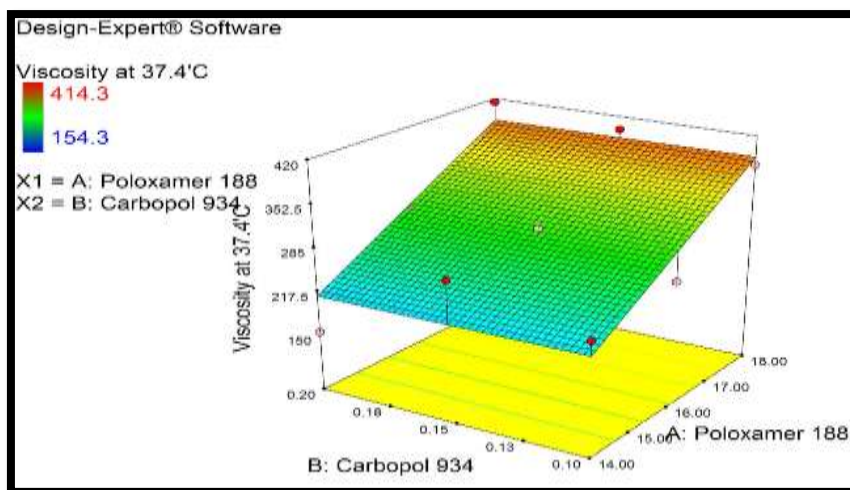

Fig 6: Surface response plot showing effect of Poloxamer 188 and Carbopol 934 on Viscosity at Room Temperature

Fig 7: Surface response plot showing effect of Poloxamer 188 and Carbopol 934 on Viscosity at 37.4°C

The figures above show the effect of concentration of Poloxamer-188 and Carbopol 934 on drug release and mucoadhesive strength. It is shown that both the independent variables have a significant effect on the dependent variables

Release Kinetics

In the present study, the release was analyzed by PCP Disso version v3 software to study the kinetics of drug release mechanism. The results showed that the factorial design batches followed zero order model kinetics.

STABILITY STUDY

Stability study of optimized F9 formulation at room temperature shown in Table 5

Table 4: Release kinetics of optimized batch

Batch (F9)	Zero order plot	First order plot	Higuchi plots	Korsmayer-peppas plot
R^2	0.9824	0.8594	0.9726	0.9681

Table 5: Stability study data for F9 batch

| Sr. No. | Observation | | Before stability testing | | During study | | | | | |
					30 Days		60 Days		90 Days	
1.	**Clarity**		Clear		Clear		Clear		Clear	
2.	**Visual appearance**		Transparent		Transparent		Transparent		Transparent	
3.	**pH**		6.36±0.16		638.±0.01		6.39±0.02		6.4±0.01	
4.	**Viscosity (rpm)**		Before Heat	After Heat	Before Heat	After Heat	Before Heat	After Heat	Before Heat	After Heat
		5	480.9	590.9	489.9	595.2	490.7	598	492.2	601..9
		10	482.1	548.9	433.9	541.9	340.7	519	276.2	439.9
		15	443.6	585.9	399.9	465.9	329.5	483	249.5	394.9
		20	439.4	482	385.9	444.5	348.3	459.1	239.2	374.9
		25	382.5	449.9	336.7	467.9	383.6	415	226.1	305.9
		30	325.9	424.3	326.7	389.2	321.9	355.2	204.2	262.4
5.	**Drug content**		99.94±0.32		98.96±0.15		98.96±0.13		98.98±0.27	

Formulations at room temperature were found to be stable up to 3 months. There is no change in drug content, pH, clarity and viscosity.

CONCLUSION

The present study was aimed to develop suitable drug delivery for the management and immediate use of Lisinopril dihydrate in the treatment of hypertension. The purpose of the study was to overcome the inherent drawbacks, associated with conventional drug delivery of Lisinopril dihydrate and will have improved bioavailability, fast therapeutic action and patient compliance with an added advantage of circumventing the hepatic first pass metabolism. Among the all formulated gels F9 was selected as the optimized formulation with respect to its evaluation parameters like pH, viscosity, drug release, mucoadhesive strength. Furthermore suitable animal studies should be carried out to established in-vitro, in-vivo correlation.

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