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# FORMULATION DEVELOPMENT AND IN VITRO EVALUATION OF IN SITU LISINOPRIL GEL FOR NASAL ADMINISTRATION

**Suvarna Bhavar*, Rahul Sarode and Dr. Ganesh Basarkar**

Department of Pharmaceutics SNJB'S Shriman Surashdada Jain College of Pharmacy, Neminagar, Chandwad, Nashik.

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***Corresponding Author**
**Suvarna Bhavar**
Department of Pharmaceutics SNJB'S Shriman Surashdada Jain College of Pharmacy, Neminagar, Chandwad, Nashik.

## ABSTRACT

Nasal route is the most suitable route for those preparations which cannot be administered orally due to the first pass metabolism to avoid this nasal route is very convenient route of administration of drug. The aim of present work was to prepare gel of lisinopril drug for intranasal route of administration, along with various excipients like gallan gum, polyethylene glycol-400(PEG-400), Mannitol, tween-80, benzalkonium chloride and distilled water. Four batches of formulations were designed, prepared and evaluated for various evaluation parameters like drug content, viscosity, mucoadhesive strength and spreadibility. Stability studies was performed and it was found that there is no significant changes in the formulation when exposed to stressed conditions like temperature and humidity for several period of time. On histopathological evaluation of optimized formulation neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after application of formulation. By seeing the results of all four formulations it was found that the batch R3 is optimized formulation.

**KEYWORD:** PEG400, IR, DSC.

## 1). INTRODUCTION

The nasal route has gained tremendous attention for systemic drug delivery by many researchers within the last few decades due to its great potential utility for drug delivery. It offers an attractive alternative for drugs that have limited oral bioavailability, are destroyed by gastrointestinal fluids, or are highly susceptible to hepatic-first pass or gut wall metabolism. Nasal drug delivery also offers the convenience and safety of being non-

invasive.[1] In addition, nasal drug administration results in quick onset of action as compared to oral, sublingual and transdermal administrations area, porous endothelial basement membrane, highly vascularized epithelial layer, high total blood flow per $cm^{3,}$ avoiding the first pass metabolism and easy access.[2] Nasal mucociliary clearance is one of the most important limiting factors for nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively rules out sustained nasal drug administration. However, mucoadhesive preparations have been developed to increase the contact time between the dosage form and mucosal layers of nasal cavities, thereby enhancing drug absorption.[3] Nasal cavity as a site for the systemic absorption of drugs has some advantages which include relatively large surface. Drugs cross the nasal mucosal membrane either transcellularly (across the cells) or paracellularly (between the cells). Lipophilic drugs are transported transcellularly by a concentration dependent passive diffusion process, by facilitated diffusion using a receptor or carrier molecule, or by vesicular transport mechanisms.[4] A significant challenge to the formulator is to overcome the protective barriers of the nasal cavity without causing permanent tissue damage. The major problems that persist with nasal solutions are cleared off rapidly from nasal cavity. The half-life of clearance for both liquid and powder formulations that are not mucoadhesive is in the order of 15–20 min. Therefore, another possible strategy is to decrease the muco-cilliary clearance by the use of mucoadhesive gel formulations to prolong the residence time at the nasal absorption site and thereby facilitate the uptake of the drug. Approache to enhance the nasal bioavailability aim at prolonging the contact time with the nasal surface by using viscosity-enhancing or in situ gelling polymer. An in situ gel drug delivery system that exhibit sol-to-gel phase transition due to change in specific physico-chemical parameters such as ionic, temperature or pH. In situ gelling systems can be classified as ion-activated systems (e.g. gellan gum and sodium alginate), temperature dependent systems (e.g. Pluronics, Tetronics and poly-methacrylates) and pH-triggered systems (e.g. Carbopol and cellulose acetate phthalate). The principal advantage of insitu gels is that they can be easily administered with accurate and reproducible dose compared to that of ordinary gels, have an advantage over ordinary gels that they can be easily installed in liquid form and are capable of prolonging the residence time of the formulation on the surface of the nasal cavity due to gelling.[5] Lisinopril (LSP) is an angiotensin-converting enzyme inhibitor used for the treatment of hypertension and congestive heart failure and to all eviate strain on hearts damaged as a result of a heart attack. LSP is slowly and incompletely absorbed after oral administration with a bioavailability of 25–30%. LSP is available only in the form of oral tablets in the market. However, this

formulation has a major disadvantage since it is incompletely absorbed from the gastrointestinal tract. To overcome the problem of incomplete absorption, low oral bioavailability and for the effective treatment of chronic hypertension, alternative long-acting formulations could be beneficial. Nasal route of administration may be a good alternative to circumvent these problems and is recognized as one of the potential route for the systemic delivery of drugs. The objective of present study is to develop the nasal in situ gel. To avoid first pass metabolism of drug, bioavaibility decreasing MCC, patient compliance gelation of gellan gum was modulated so as physiological ion content after using simulated nasal fluid.

## 2). MATERIAL AND METHOD

### Material

Lisinopril was a gift sample from Hetro drug limited, India. Gellan gum was obtained as gift sample from CPKelco, Singet Chemical Corporation Pvt. Ltd Mumbai, India. All other material and solvent obtained from commercial sources were of analytical grade.

### Preparation of in situ gelling systems

Accurately weight quantity of gellan gum 0.1-0.4%w/v) was dispersed in distilled water. The dispersion were then stirred for 20 min at 85-90°c using magnetic stirrer and then cooled to room temperature. Mucoadhesive agent was added slowly with stirring. D-mannitol(5%), benzalkonium chloride(0.002%w/v), penetration enhancer were also added simultaneously. Finally lisinopril was added with stirring. The pH of all formulation was in the range 4.5-6.5. formulation was filled in amber colored glass vials, capped with rubber closure and sealed with aluminium caps. Formulation was stored in refrigerator (4-8°C) until use.[6,7]

**Table 1:- Composition of various ion-activated mucoadhesive nasal in situ gel formulations**

| Sr.No | Ingredients | R1 | R2 | R3 | R4 |
|---|---|---|---|---|---|
| 1 | Lisinopril(mg) | 10 | 10 | 10 | 10 |
| 2 | Gellan gum | 0.1 | 0.2 | 0.3 | 0.4 |
| 3 | PEG400 | 6 | 6 | 6 | 6 |
| 4 | Mannitol | 5 | 5 | 5 | 5 |
| 5 | Tween 80 | 1 | 1 | 1 | 1 |
| 6 | Benzalkonium chloride | 0.002 | 0.002 | 0.002 | 0.002 |
| 7 | Distilled water | q.s | q.s | q. s | q. s |

## 3). PREFORMULATION STUDY

### Determination of λ max of Lisinopril

The stock solution of 100µg/ml was prepared by dissolving Lisinopril equivalent to 10 mg lisinopril in 100 ml of distilled water. Then, spectrum of this solution was obtained by scanning between 400 and 200 nm using UV visible spectrophotometer (Shimadzu UV-1800, Japan). The absorption maximum wavelength was determined.

**Table 2:-Calibration curve data of lisinopril in phosphate buffer pH 6.2**

| Sr. No. | Concentration(µg/ml) | Absorbance |
|---|---|---|
| 1 | 10 | 0.101±0.035 |
| 2 | 20 | 0.203±0.025 |
| 3 | 30 | 0.300±0.025 |
| 4 | 40 | 0.399±0.050 |
| 5 | 50 | 0.495±0.051 |

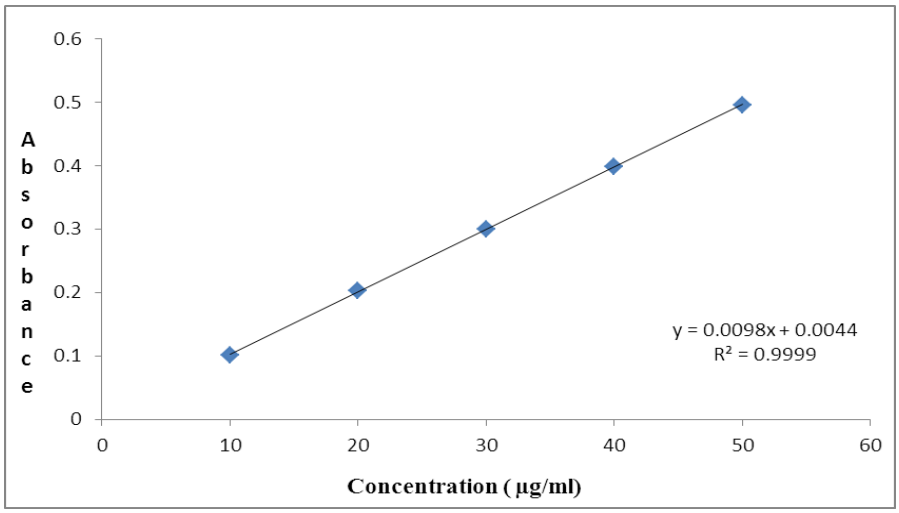


**Figure 1:- Standard calibration curve of lisinopril in phosphate buffer pH 6.2**

### Optimum concentration of deacetylated gellan gum for in situ gelation

Preliminary studies were carried out using different concentrations of gellan gum as shown in Table 1. The simulated nasal fluid was prepared by dissolving sodium chloride(2.1925 g), calcium chloride (0.145 g) and potassium chloride(0.745 g) into 250 ml of double distilled water. The gelling concentration of deacetylated gellan gum was optimized on the basis of minimum possible concentration which would produce gelation with minimum viscosity. Gelation studies were carried out using simulated nasal fluid (pH 6.4 ± 0.1) at 34_±1°C. Preliminary studies revealed.[4,8]

**Table 3:-gelation studies with deaceylated gellan gum**

| Sr.No. | Concentration of deacylated gellan gum (%w/v) | Degree of gelation |
|---|---|---|
| 1 | 0.1 | No Gelation (-) |
| 2 | 0.2 | Weak gelation, dissolved rapidly (+) |
| 3 | 0.3 | Immediate gelation remains for few hrs(++,less stiff gel) |
| 4 | 0.4 | Immediate gelation remains for extended periods(+++,stiff gel) |

**Clarity**

Abbe's refractometer has been used to measure the refractive index of liquids. The clarity of solutions, that is formulations before gelling, was determined in term of refractive index using Abbes refractometer. The refractometer was calibrated with water as reference standard. The refractometer scale was adjusted in such a way that the cross wire of the telescope was exactly on the boundary between the bright and dark regions. The procedure was repeated for all formulations F1 to F4 and the results were compared with refractive index of water (1.33),[4]

**PH**

Digital pH meter (Chemline, CL180, India) was calibrated by using pH buffer of 4 and 7. Twenty milliliter of each formulation was taken in beaker and glass electrode was sufficiently dipped into the samples of formulations. Then, pH of the solution was determined in triplicate.[4]

**Drug content**

Drug contents of formulations were determined in triplicate by using double beam UV visible spectrophotometer(Shimadzu UV-1800). One milliliter of formulation was taken in capacity of 10 ml volumetric flask, diluted with double distilled water and volume adjusted to10 ml. One milliliter quantity from this solution was again diluted with 10 ml of double distilled water. Finally, the absorbance of prepared solution was measured at 226 nm by using UV visible spectrophotometer.[4]

**Viscosity**

The viscosity measurements were carried out by using Brookfield viscometer RV. The viscosity measurements were performed using small volume adapter. The temperature

sensing probe was lowered in gel and temperature of gel was recorded. Viscosity at 32–34$^0$C temperatures was noted.[4]

**Mucoadhesive strength**

It is the force required to detach the formulation from nasal mucosal tissue. The mucoadhesive force, the detachment stress of the formulation was determined using a modification of the mucoadhesive force measuring device. The modified balance technique using two-glass vials and sheep nasal mucosa was used. A nasal mucosa with thickness of 0.6mm and surface area 2.835 cm$^2$ was cut from the olfactory region of sheep nasal cavity and instantly secured with the mucosal side out onto each glass vial using a thread. The vials were stored at 32–34$^0$C for 10 min. One vial was attached to one side of balance and 0.5 ml of gel sample was placed between the two mucosal membranes attached to the bottom of the vials. The minimum weight of water required to break the mucosal adhesion was measured. Mucoadhesive Strength(dynes=cm²) mg=A.

Where m is weight required for detachment in g, g is acceleration due to gravity (980 cm/s$^2$) and A is surface area of mucosa exposed (cm$^2$).[4]

**Spread ability**

Spread ability is the area traveled per unit time (cm²/min) by the gel formulation. Whatmann filter paper (0.45 mm) was used for determination of spread ability of solution formulations F1 to F4. A 1-ml graduated pipette with rubber bulb was clamped vertically to the stand in such a way that the tip of pipette was at 2 cm above the horizontal surface of round shape filter paper. A 0.1-ml sol formulation was dropped at center of filter paper. At fixed time interval, 20 s, the surface area covered by the formulation was measured.[4]

**Ex vivo permeation studies**

Fresh nasal mucosa from olfactory region was carefully removed from the nasal cavity of sheep obtained from the local slaughter house. Nasal mucosa was inserted into phosphate buffer pH 6.4. Tissue samples were placed on diffusion cells immediately. Phosphate buffer solution pH 6.4 at 34$^0$C was added to the acceptor chamber. Formulation equivalent to10 mg of lisinopril was placed in the donor chamber. At predetermined time points, 1 ml samples were withdrawn from the acceptor compartment, replacing the sample volume with phosphate buffer pH 6.4 after each sampling, for a period of 10 h. The samples withdrawn were filtered and used for analysis. Blank samples (without lisinopril) were run

simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using a UV-visible Spectrophotometer at 206 nm (Linearity range=1 to 8µg/ml, $R^2$=0.998).[4]

**Stability study**

Stability studies were carried on in situ gel formulation according to ICH (International conference on Harmonization) guidelines. A sufficient quantity of in situ gel in amber colored sealed vials was stored in desicator containing saturated solution of sodium chloride, which gave a relative humidity 75±5%. The desicator was placed in hot air oven maintained at 40±2°C.[6]

**4). RESULT**

Lisinopril exhibits max at 206.0nm .Linearity was observed in range of 10-100µg/ml with the $r^2$ value of 0.998.

**FTIR**

FTIR Studies were carried out on pure drug as well as its combination with selected polymer. IR characteristics of lisinopril with the polymer resemble almost the IR structural characteristics of pure drug indicated the compatibility between the drug and polymers. The infrared spectra of lisinopril and physical mixture of formulation containing drug, gellan gum shown in figure.

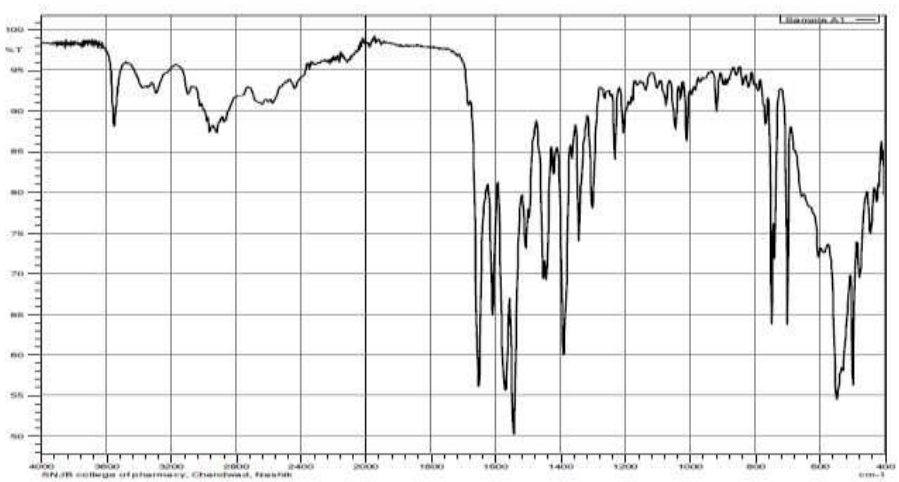

**Figure2:-IR spectrum of lisinopril**

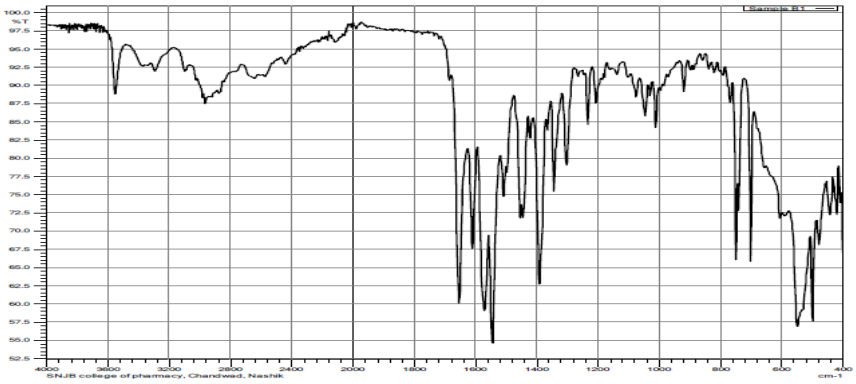

**Figure3:- IR Spectrum of the drug:gellan gum**

**DSC**

DSC thermo gram were recorded for pure Lisinopril and physical mixture of drug and polymer (Figure. In both cases it was observed that the characteristics endotherm (Corresponding to melt of drug) did not shift appreciably, suggesting the lack of any interaction between the drug and excipient.

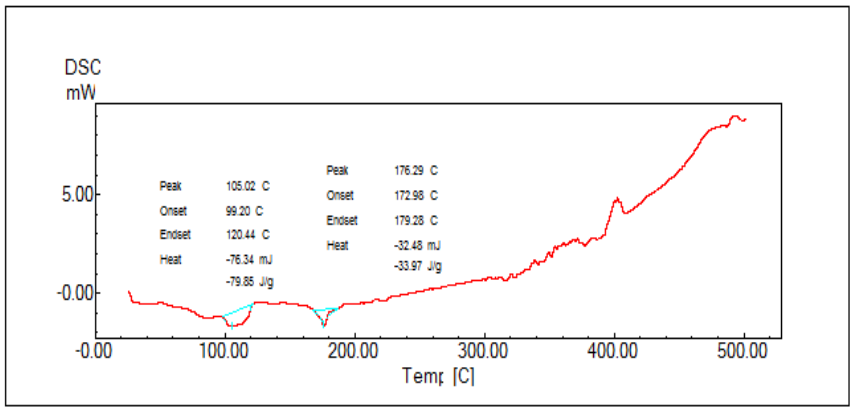


**Figure4:- DSC thermo gram of Lisinopril**

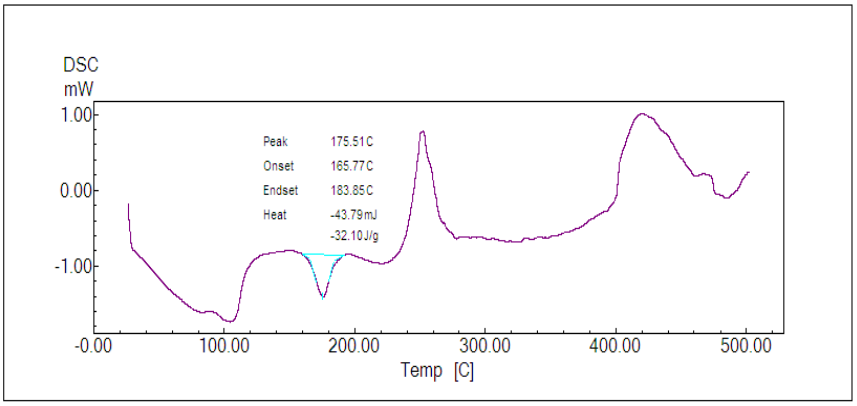


**Figure5:- DSC thermo gram of physical mixture of drug and gellan gum**

**PH of formulation**

The pH of all formulation was found to be in the range of 4.66 to 6.45 which is in the nasal (4.5–6.4) pH range.

**Drug content**

Table shows that the percent drug content for all formulations of the drug contents were found in the range of 96–101%. The tests were carried out in triplicate.

**Refractive index**

Refractive index was close to refractive index of water, that is 1.33. Hence, all formulations shown in Table were clear in appearance.

**Viscosity**

Table shows the viscosity values obtained for all formulation using Brookfield viscometer. Formulation F3 containing gellan gum 0.3% w/v exhibited optimum viscosity as well as mucoadhesive strength.[9]

**Spreadability**

It is very important for in situ gel to have suitable spreadability to administer easily and to spread easily on nasal mucosa without leakage after administration. Table shows the data of spreadability measurement. Formulation F3 shows maximum spreadability due to more surface area covered by in situ gel after placing on filter paper.

**Table 4:- Result of optimized batch**

| Batch code | Visual appearance | Refractive index | PH | %Drug content |
|---|---|---|---|---|
| F1 | Colorless solution | 1.366±0.001 | 5.690±0.044 | 99.551±0.220 |
| F2 | Colorless solution | 1.362±0.002 | 5.720±0.126 | 99.662±0.243 |
| F3 | Colorless solution | 1.335±0.002 | 5.45±0.0027 | 98.93±0.182 |
| F4 | Colorless solution | 1.337±0.001 | 5.680±0.084 | 100.502±0.196 |

**Table 5:- Result of optimized batch**

| Batch code | Spread ability | Mucoadhesive strength(dyne/cm$^2$) | Viscosity (cps) | Drug release(%) |
|---|---|---|---|---|
| F1 | 21.195±0.166 | 2030.5±57.21 | 60 | 99.833±0.274 |
| F2 | 18.462±0.114 | 2429.8±152.30 | 166±5.77 | 98.770±0.277 |
| F3 | 13.564±0.321 | 2729.36±152.90 | 250±10 | 97.51.172±0.309 |
| F4 | 15.918±0.185 | 2812.83±189.50 | 346.66±5.77 | 90.63±0.216 |

**In vitro drug release study**

The in vitro release was carried out for all formulation using phosphate buffer pH 6.4 as medium. The in vitro drug release study revealed that the release rate depended on gellan gum concentration. The higher the gellan gum concentration, the lower the rate of drug release. The data of these studies are presented in the form of graph. The formulation F3 containing 0.3% w/v gellan gum and 6% w/v PEG 400 showed 97.34% drug release within 10 h which was satisfactory. In general, it was found that drug release for all formulations, F1 to F4, was more than 90% after 10 h. This is shown in Figure.

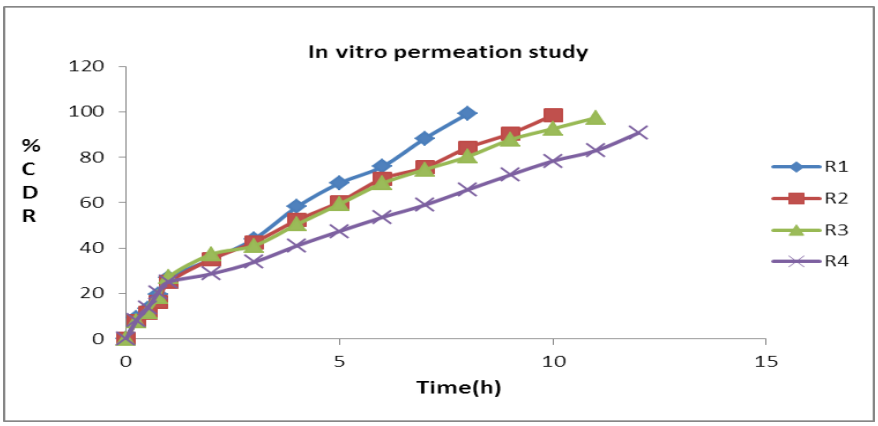


**Figure6:-In vitro drug release profile of formulation R1 to R4**

**Ex vivo permeation studies**

Optimized formulation(R3) and R0 subjected for ex vivo permeation studies using the sheep nasal mucosa. The permeation of lisinopril from formulation R3 without tween 80 was slow (78.37% in 10 h). However, permeation of drug increased to 88.50% in 10h for the formulation R3which contains Tween 80(1%). The increase in permeation of lisinopril could be attribute to penetration enhancement action of Tween 80.

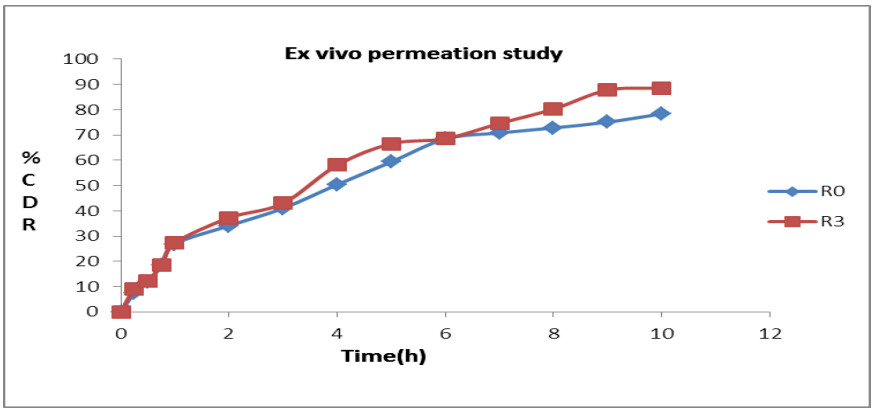


**Figure7:- Ex vivo permeation profile of optimized batch and P0**

**Histopathological evaluation of nasal mucosa**

The microscopic observation indicates that the optimized formulation has no any significant effect on the microscopic structure of mucosa as shown in Figure. Neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after application of formulation and buffer pH 6.4.[10]

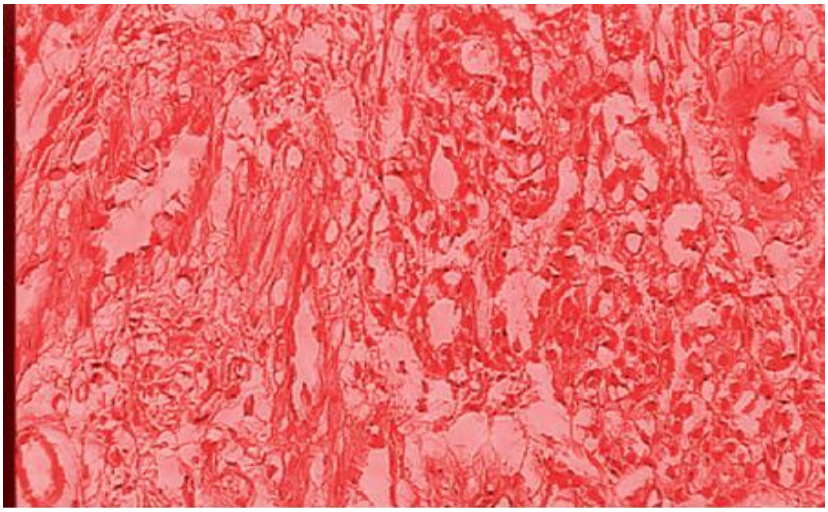

**Figure.8**

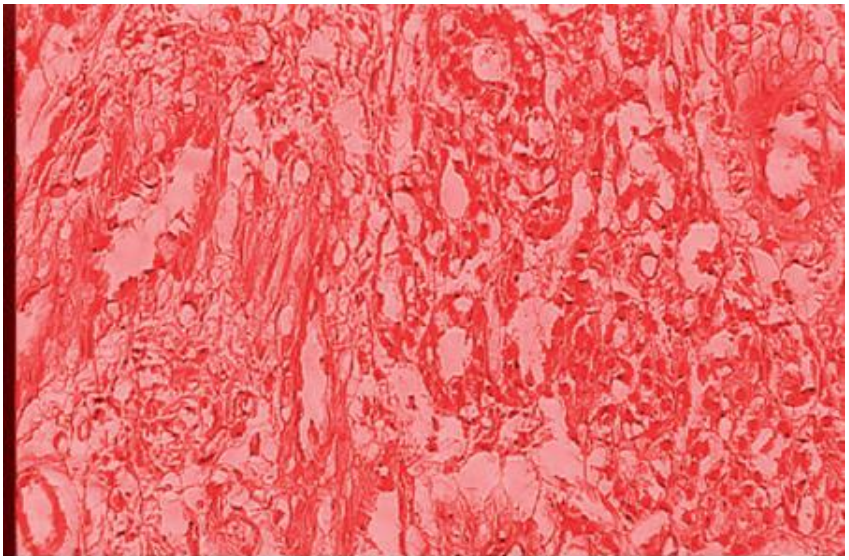

**Figure.9**

**Stability study**

The stability study of optimized formulation(R3) revealed that no significant changes in the physical parameter when stored at temperature and humidity conditions at 40±2°C and 75±5°C RH. No significant variation in the gelation property, viscosity, pH, drug content and

in vitro mucoadhesive strength over a period of three months. The stability data of optimized formulation is shown in table.

**Table 6:- Stability study of optimized formulation at 40±2°Cand 75±5%RH**

| Time period | Physical properties | Viscosity (cps) | PH | Drug content |
|---|---|---|---|---|
| 1 month | No change | 81.11±1.92 | 5.96±0.23 | 99.89±0.51 |
| 2 month | No change | 71.10±6.93 | 5.79±0.23 | 98.89±0.37 |
| 3 month | No change | 61.10±5.09 | 5.57± 0.51 | 98.89±0.57 |

## 5). DISCUSSION

The purpose of present study was to develop a novel in situ mucoadhesive gel of lisinopril for intranasal administration. Lisinopril ACE inhibiter, a powerful hypertensive belongs to BCS class III, which has oral bioavailability of 25-30% due to hepatic first pass metabolism. In situ gel offers various advantages like ease of preparation and administration, accuracy of dosing, improve bioavailability, decrease nasal mucociliary clearance and improve patient compliance.

Preformulation study was carried out for identification of drug and drug excipient compatibility study by isothermal stress testing, DSC and IR. Dose of formulation was determined from in vitro diffusion study. Preliminary trial were preformed on placebo in situ gel for selection of ion induced polymer concentration. The prepared in situ gels were evaluated for in vitro gelatin, viscosity and in vitro mucoadhesive strength. In situ gels were formulated using penetration enhancers like Tween 80 and subjected for in vitro drug release study for selection of suitable penetration enhancer.

Prototype formulation was prepared using gellan gum as ion induced polymer, Tween 80 as a penetration enhancer, D- mannitol as osmolality adjusting agent, benzalkonium choride as preservative and distilled water as a vehical. Prototype formulation were evaluated for in vitro gelation, viscosity of sol-gel state, pH, drug content, in vitro mucoadhesive strength and in vitro drug release study. Stability study of optimized batch (F3) was carried as per ICH guidelines i.e. accelerated stability study at 40±2°c and 75±5% RH. Optimized formulation was also subjected for ex vivo permeation and histopathological study using sheep nasal mucosa.

From result of present work, it can be conclude that, Drug excipient compatibility was revealed that no significant interaction between drug and excipients, therefore drug was

compatible with excipient used for formulation development from preliminary trails, 0.3% gellan gum showed optimum gelation. permeation enhancement of lisinopril from in situ gel was investigated in Tween 80 (1%w/v). prototype formulation were prepared by varying concentration of gellan gum and batch no (F3) showed optimum gelation, pH in the range of nasal cavity, satisfactory viscosity for spraying as droplet, drug content within limit, good mucoadhesive strength and better drug release. Ex vivo permeation study was investigated that increases in permeation across sheep nasal mucosa using Tween 80 and no sign of remarkable destructive effect of optimized formulation on mucosa Optimized formulation was also retained stability at accelerated condition for period of 3 months.

## CONCLUSION

In situ gel approach can be utilized for needleless administration which may result in development of patient friendly drug delivery system. it may be prolong the residence time of the formulation in the nasal cavity due to mucoadhesive and gel structure by minimizing mucociliary clearance. Thus, in situ gel for nasal delivery is available alternative to other routes of administration in order to improve the therapeutic efficacy of lisinopril.

## 6). ACKNOWLEDGEMENT

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