



DESIGN AND EVALUATION OF BIOADHESIVE BUCCAL DRUG DELIVERY SYSTEMS CONTAINING LESINURAD

Pharmaceutical

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ABSTRACT

Aim : The intent of present work was concerned with formulation and evaluation of bioadhesive buccal drug delivery of lesinurad tablets, which is plentifully metabolized by liver and avoiding first pass metabolism and prolonging duration of action. Lesinurad is a urate transporter inhibitor for treating hyperuricemia associated with gout. **Materials and Methods:** Lesinurad tablets were prepared by direct compression method using bioadhesive polymers such as hydroxypropyl methylcellulose K 200M, Carbopol 940 and sodium alginate in different ratios and ethylcellulose as backing layer. The formulations were characterized for physicochemical parameters, in vitro release studies. The physicochemical compatibility of drug and polymers was studied by FT-IR spectroscopy. **Results and Discussion:** The formulations were tested for bioadhesive strength, ex vivo residence time, swelling time and in vitro dissolution studies and ex vivo permeation studies, bioadhesive strength, surface PH. Optimized formulation (F8) showed 92 % in vitro release in 8 h and 65.8% permeation of drug through porcine buccal mucosa and followed fickian release mechanism with zero order kinetics. This product was more comfortable to the user due to absence of erosion, faster hydration rate and less viscosity of surrounding environment FTIR studies of optimized formulation showed no evidence of interaction between the drug and polymers. To conclude that the formulated unidirectional, bilayered, bioadhesive buccal tablet for lesinurad using HPMC as mucoadhesive agent is superior to oral conventional tablets, as it has the potential to bypass the first pass metabolism and improve the bioavailability of lesinurad. In vivo mucoadhesive behaviour of optimized formulation was performed and parameters were evaluated.

KEYWORDS

HPMC, Swelling, bioadhesive buccal tablets, evaluation, lesinurad, formulation, bioavailability.

INTRODUCTION

Buccal administration of drugs provide a convenient route of administration for both systemic and local drug actions. However, the preferred site for retentive oral transmucosal delivery systems and for sustained and controlled-release delivery devices is the buccal mucosa, mainly because of the difference in permeability characteristics between the two regions and the buccal mucosa's expanse of smooth and relatively immobile mucosa.^[1] Recently, considerable attention has been focused on the development of alternative drug delivery systems for proteins and peptide drugs. As the peroral administration has disadvantages such as the hepatic first pass metabolism and enzymatic degradation within the gastrointestinal tract, proteins and peptides are usually not suitable for peroral administration and are mostly delivered by parenteral.^[2] Nasal, ocular, vaginal, rectal and buccal mucosal membranes have been evaluated as potential alternative routes for peptide absorption. Buccal delivery of drugs provides an attractive alternative to the oral route of drug administration, particularly in overcoming deficiencies associated with the latter mode of administration. Problems such as high first-pass metabolism and drug degradation in the harsh gastrointestinal environment can be circumvented by administering the drug via the buccal route.^[3,4] Moreover, buccal drug absorption can be promptly terminated in case of toxicity by removing the dosage form from the buccal cavity. It is also possible to administer drugs to patients who cannot be dosed orally to prevent accidental swallowing. Therefore mucoadhesive dosage forms were suggested for oral drug delivery which include adhesive tablets,^[5,6] adhesive gels,^[7] adhesive patches.^[8] Lesinurad is an oral uric acid transporter 1 (URAT1) inhibitor indicated for the treatment of hyperuricemia associated with gout. It reduces serum uric acid concentration through the inhibition of URAT1, an enzyme responsible for reuptake of uric acid from the renal tubule, and OAT4, another uric acid transporter associated with diuretic-induced hyperuricemia.

Lesinurad {[5-bromo-4-(4-cyclopropyl)naphthalen-1-yl]-4H-1,2,4-triazol-3-yl}sulfanyl]acetic acid under the brand name Duzallo indicated for the treatment of gout-related hyperuricemia in patients with uncontrolled gout. The peak plasma concentration is reached with in 1.5h. The bioavailability of lesinurad is about 9% and half life is 6 hr and is widely distributed through out the body and 98% of drug binds to plasma proteins. It is extensively metabolised in liver to undergoes oxidative metabolism mainly via the polymorphic cytochrome P450 CYP2C9 enzyme. Plasma exposure of metabolites is minimal (<10% of unchanged lesinurad); both metabolites are pharmacologically inactive leading to lower bioavailability. So, lesinurad is selected as model drug for investigation because of its suitable properties like half-life of 5 hrs, optimum partition coefficient (159) and molecular weight (404.28) make it suitable form administration by buccal route

to overcome extensive first-pass metabolism. A suitable buccal drug delivery system should possess good bioadhesive properties. So, that it can retain in oral cavity for desired duration and localize the dosage form in a specific region and control the release rate of drug.

EXPERIMENTAL (MATERIALS AND METHODS):

Materials

Lesinurad was generously gifted by Orchid Pharma Ltd, Chennai. HPMC K4M was gifted by signet chemical corporation, Mumbai. Sodium alginate, Mg.stearate and Carbopol were purchased from S.D fine chemicals. Mumbai, India. All other chemicals and reagents used were of analytical grade.

Methods:

Pre formulation studies

Drug-excipient compatibility studies

Fourier Transform Infrared spectroscopic studies

A Fourier Transform – Infra Red spectrophotometer was used to study the non-thermal analysis of drug–excipient (binary mixture of drug : excipient 1:1 ratio) compatibility. The spectrum of each sample was recorded over the 4000-450cm⁻¹. Pure drug of Lesinurad, Lesinurad with physical mixture (excipients) compatibility studies were performed.

Calibration curve of Lesinurad:

100 mg of Lesinurad was dissolved in small amount of phosphate buffer and make the volume up to 100 mL with phosphate buffer pH 6.8, from this primary stock (1mg/mL), 10 mL solution was transferred to another volumetric flask made up to 100 mL with Phosphate buffer pH 6.8. From this secondary stock 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8mL was taken separately and made up to 10 mL with phosphate buffer pH 6.8 to produce 4, 8, 12, 16, 18, 20, 24, 28 µg/mL respectively. The absorbance was measured at 260 nm using a UV spectrophotometer.

Solubility studies:

Lesinurad solubility studies in phosphate buffer, pH 6.8 was determined by phase equilibrium method. An excess amount of Lesinurad was taken in 20 mL vial containing 10 mL of phosphate buffer, pH 6.8. These vials were closed with rubber caps and agitated at room temperature for 24 h using rotary shaker (Remi Instruments, Mumbai, India). The solution was filtered through a 0.2 µm whatman filter paper after 24 h. The amount of drug solubilized was estimated by measuring absorbance at 260 nm using a UV spectrophotometer (Systronic PC Based Double Beam Spectrophotometer 2202, Ahmedabad, India). The studies were repeated in triplicate (n=3) and mean was calculated.

Evaluation of Pre compression Blend

Angle of repose

Angle of repose and Carr's compressibility index were used to determine the powder flow properties. Angle of repose (θ) was measured by fixed funnel method and it was determined by given formula:

$$\tan \theta = h/r$$

where ' θ ' was angle of repose, 'h' and 'r' were height and radius of the cone of powder.

The tendency of a powder to be compressed can measured by Carr's index and determined using given below formula:

Bulk density

Density is defined as weight per unit volume. Bulk density, ρ_b , is defined as the mass of the powder divided by the bulk volume and is expressed as gm/cm^3 . The bulk density of the powder primarily depends on particle size distribution, particle shape and tendency of particles adhere together. Bulk density is very important in the size of containers needed for handling, shipping and storage of raw material and blend. It is also important in size of blending equipment. 30g of powder blend introduced into a dry 100mL cylinder, without compacting. The powder was carefully leveled without compacting and unsettled apparent volume (V_a) was read (Lachman 2009).

The bulk density was calculated using the formula:

$$\rho_b = M/V_a$$

Where, ρ_b = Apparent bulk density

M = Weight of the sample

V = Apparent volume of powder

c) Tapped density

After carrying out the procedure as given in the measurement of bulk density the cylinder containing the sample was tapped using a suitable mechanical tapped density tester that provides a fixed drop of 14 ± 2 mm at a nominal rate of 300 drops per minute, the cylinder was tapped 500 times initially followed by an additional tap of 750 times until difference between succeeding measurement is less than 2% and then tapped volume, V_f was measured to the nearest Graduated unit. The tapped density was calculated, in gm per mL, using the formula:

$$\rho_{\text{tap}} = M/V_f$$

Where, ρ_{tap} = Tapped density

M = Weight of the sample

V_f = Tapped volume of Powder

Powder Compressibility

The compressibility index (Carr's index) is a measure of the propensity of a powder to be compressed. It is determined from the bulk and tapped densities. In theory the less compressible material the more flowable it is. As such it is measure of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the compressibility index which is calculated using the following formula:

$$\text{Carr's index} = [(\rho_{\text{tap}} - \rho_b) / \rho_{\text{tap}}] \times 100$$

Where, ρ_b = bulk density

ρ_{tap} = tapped density

Hausner's ratio

It is the ratio of tapped density and bulk density. Hausner found that this ratio was related to interparticle friction and as such could be used to predict powder flow properties. Generally a value less than 1.25 indicates good flow properties, which is equivalent to 20% of Carr's Index.

Hausner's ratio = Tapped density/Bulk density

Mucoadhesive Tablets Preparation:

Lesinurad was mixed manually with different ratios of carbopol-934, HPMC, sodium alginate and microcrystalline cellulose act as a diluent, PVP K-30 as dry binder (Table-1). The blend was lubricated with magnesium stearate for 3-5 min and then compressed into tablets by direct compression method using 8mm flat faced punches, (Rimek Minipress, Karunavati Eng. Ltd, Ahmedabad). The mass of tablets were determined using Digital balance (Shimadzu, Japan) and thickness of tablets with a digital Screw Gauge (Mitatyo, Japan).

Table 1: Composition of buccal tablet

INGREDIENTS	FORMULATION CODE								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Lesinurad	200	200	200	200	200	200	200	200	200
Sodium Alginate	100	150	200		-	-	-	-	-
Carbopol 940	-	-	-	100	150	200	-	-	-
HPMC K 200M	-	-	-	-	-	-	100	150	200
PVP K30	10	10	10	10	10	10	10	10	10
Magnesium Stearate	2	2	2	2	2	2	2	2	2
Talc	1	1	1	1	1	1	1	1	1
MCC	137	87	37	137	87	37	137	87	37
Total Tablet Weight	450	450	450	450	450	450	450	450	450

API- Active pharmaceutical ingredient (Drug), CP- Carbopol 940, HPMC - Hydroxy propyl methyl cellulose, PVP K 30 – Poly Vinyl Pyrolidone, MCC – Micro Crystalline Cellulose

Evaluation of buccal tablets:

Physicochemical characterization of tablets:

The prepared Lesinurad buccal tablets were studied for their physicochemical properties like weight variation, hardness, thickness, friability and drug content (Indian pharmacopoeia, 2007).

Assay of Lesinurad:

Twenty tablets were taken and powdered; powder equivalent to one tablet was taken and allowed to dissolve in 100ml of water on a rotary shaker overnight. The suspension was centrifuged and supernatant liquid was collected and the absorbance was measured using UV visible Spectrophotometer 119 (Systronics, Naroda, Ahmedabad) at 260 nm.

In-Vitro Release Studies :

The drug release rate from buccal tablets was studied using the USP (II) dissolution test apparatus (Labindia dissolution test apparatus Disso 2000). The assembly is kept in a jacketed vessel of water maintained at 37 ± 10 C. Buccal tablet was made to stuck on bottom of the flask (so as to allow one sided release from the tablet). The beaker is filled with 250ml of mixed phosphate buffer pH 6.8. The vessel maintained at 50 rpm under stirring conditions by means of paddle fabricated for purpose in dissolution apparatus. At various intervals of time, samples were withdrawn and filtered through whatmann filter paper no.42. It is replaced immediately with equal amount of fresh buffer. The samples are then analyzed U.V. spectrophotometrically at 260 nm up to 8 hours.

In-Vitro Swelling Studies:

The tablets of each formulation were weighed individually (W_1) and placed separately in Petri-dishes containing 2% Agar gel. At regular intervals (1, 2, 3, 4, 5, 6, 7 and 8 hours) the tablets were removed from Petri dishes and excess water removed carefully using filter paper. The swollen tablets were re-weighed (W_2); the swelling index of each formulation calculated by using this formula.

$$\text{Swelling Index (S.I.)} = W_1 - W_2 / W_1$$

Thickness :

The thickness of tablets was determined using digital caliper (Aerospace, India). Ten individual tablets were measured and the average thickness was calculated.

Weight variation:

Weight variation test was carried out for ten tablets of each batch using an electronic balance (Shimadzu, Aux*200, Japan) and average values were calculated.

Hardness

Hardness for three tablets of each batch was measured by using Monsanto hardness tester and average values were calculated.

Ex vivo residence time

The ex vivo residence time of buccal tablets was determined using locally modified USP disintegration apparatus. The disintegration medium was 800mL phosphate buffer, pH 6.8 maintained at $37 \pm 0.2^\circ\text{C}$. The buccal membrane of porcine was tied to the surface of a glass slide vertically attached to the apparatus. One surface of tablet was hydrated using 0.5 mL of phosphate buffer, pH 6.8 and then the hydrated surface was brought in contact with the mucosal membrane. The glass slide was vertically fixed to the apparatus and allowed to run in such way that the tablet completely immersed in the buffer solution at the lowest point and was out at the highest point. The time taken for complete erosion or displacement of the tablet from the mucosal surface was noted. The experiments were performed in triplicate ($n=3$) and mean of triplicate was determined.

In vitro drug release studies

The porcine buccal membrane was mounted between the donor and receptor compartment of the standard Franz diffusion cell and the acceptor compartment volume of 20 ml. A semipermeable membrane (buccal mucosa) was clamped between the donor and acceptor compartments. The phosphate buffer in the acceptor compartment was continuously stirred at 600 rpm using a magnetic stirrer. The tablet was placed in to the donor compartment and was wetted with 1 ml of phosphate buffer. The amount of drug permeated through the membrane was determined by removing aliquots from the acceptor compartment and by replacing the same volume of buffer. The flux (J) through the membrane was calculated by using the equation. The values are shown in table 9.

$$J = dQ / A dt$$

Where, J is flux ($\text{mg h}^{-1} \text{cm}^2$);

dQ / dt is the slope obtained from the steady-state portion of the curve and A is the area of diffusion (cm^2).

Surface pH study

The bioadhesive tablet was allowed to swell by keeping it in contact with 1mL of distilled water for 2 h at room temperature. The pH was measured by bringing the pH-meter electrode, in contact with the surface of the tablet and allowing it to equilibrate for 1 min.^[11]

Moisture absorption

Agar (5%, m/V) was dissolved in hot water and transferred into Petri dishes and allowed to solidify. Six buccal tablets from each formulation were placed in a vacuum oven overnight prior to the study to remove moisture, if any, and laminated on one side with a water impermeable backing membrane. They were then placed on the surface of the agar and incubated at 37°C for one hour. Then the tablets were removed and weighed and the percentage of moisture absorption was calculated.^[12]

Ex vivo bioadhesion strength

Bioadhesion strength of tablets were evaluated using a microprocessor based on advanced force guage equipped with a motorized test stand (Ultra Test Tensile strength tester, Mecmesin, West Sussex, UK) according to method describe as it is fitted with 25kg load cell, in this test porcine membrane was secured tightly to a circular stainless steel adaptor and the buccal tablet to be tested was adhered to another cylindrical stainless steel adaptor similar in diameter using a cyanoacrylate bioadhesive. Mucin 100 μL of 1 %w/v solution was spread over the surface of the buccal mucosa and the tablet immediately brought in contact with the mucosa. At the end of the contact time, upper support was withdrawn at 0.5mm/sec until the tablet was completely detached from the mucosa. The work of adhesion was determined from the area under the force distance curve^[13]

Friability:

Friability is the measure of a tablet's ability to with stands both shock and abrasion without crumbling during the handling of manufacturing, packing, shipping and consumer use. The weight of 10 tablets was noted and placed them in Roche friabilator. The device subjects the tablets to the combined effect of shock and abrasion by utilizing a plastic chamber, which revolves at 25 rpm, dropping the tablets a distance of 6 inches with the revolution. The pre-weighed tablet sample is removed after 100 revolutions, dusted and reweighed. Tablets that loose less than 0.5 to 1 percent in weight are generally considered acceptable.

$$\text{Friability (\%)} = \frac{\text{Initial wt. of 10 tablets} - \text{Final wt. of 10 tablets} \times 100}{\text{Initial wt. of 10 tablets}}$$

FTIR Studies:

IR spectra for pure drug Salbutamol, Salbutamol bilayered buccal tablets F8were recorded in a Fourier transform infrared (FTIR) spectrophotometer (FTIR 1615, Perkin Elmer, USA) with KBr pellets.

Kinetic study:

To analyze the in vitro release data various kinetic models were used to describe the release kinetics.

1. Zero – order kinetic model – Cumulative % drug released versus time.
2. First – order kinetic model – Log cumulative percent drug remaining versus time.
3. Higuchi's model – Cumulative percent drug released versus square root of time.
4. Korsmeyer equation / Peppas's model – Log cumulative % drug released versus log time.

Stability study:

The fabricated lesinurad buccal tablets formulations were subjected for stability study. The stability study was carried out according to ICH guidelines at $40 \pm 2^\circ\text{C} / 75 \pm 5\%$ RH for three weeks. For stability study, the tablets were sealed in aluminum packing coated inside with polyethylene. These sample containers were placed in desiccators maintained at 75% RH. The product was evaluated for drug content, bioadhesive strength and swelling index study. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of a variety of environmental factors such as temperature, humidity and light, and enables recommended storage conditions.

RESULTS AND DISCUSSIONS

Preformulation study:

Preformulation studies are primarily done to investigate the physicochemical properties of drug and to establish its compatibility with other excipients used.

FTIR Compatibility Studies:

FTIR spectra of pure drug and formulation with other ingredients were recorded. The FTIR Spectra of pure Lesinurad drug and polymer was compared with the FTIR spectrum of drug and optimised in the figures, respectively.

Fig 1: FTIR studies of pure drug Lesinurad

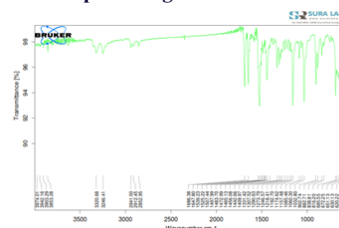


Figure 2: Pure Drug (Lesinurad) +HPMC K 200 M FTIR

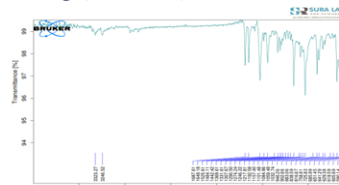


Figure 3: Drug (Lesinurad) + Sodium Alginate FTIR

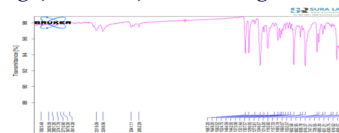


Figure 4: Drug (Lesinurad) + Carbopol 940 FTIR

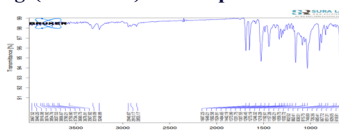
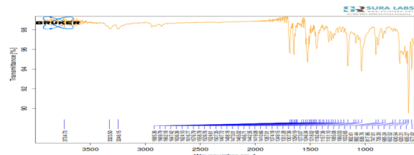


Figure 5: FTIR compatibility studies of optimized formulation**FTIR INTERPRETATION:**

There was no appearance or disappearance of any characteristic peak in the FTIR spectrum of drug and the polymers used. This shows that there is no chemical interaction between the drug and the polymers

S. No	Functional Group	Range	Lesinurad	Lesinurad + Sodium Alginate	Lesinurad + Carbopol 940	Lesinurad + HPMC K 200M	Optimized formulation
1.	Alcohols O-H stretch	~3650 or 3400-3300	3320.68	3319.09	3319.09	3323.27	3323.50
2.	Amines C-N Stretch (aryl)	1360-1250	1273.79	1273.61	1273.19	1274.24	1273.07
3.	alkyl halides C-Br stretch	650-510	577.05	575.99	576.92	575.67	577.02
4.	Aromatics C-H bend	850-800	838.81	838.78	838.51	838.59	838.76
5.	O-H bend	1460-1400	1442.66	1442.05	1442.19	1442.42	1442.35
6.	Aromatics C-H bend	~880	882.71	882.95	882.41	883.06	882.44

Table 2: Solubility studies of Lesinurad

Medium	Amount present (µg/mL)
Distilled water	22.57
Phosphate buffer pH 6.8	89

Calibration Curve of Lesinurad

Standard graph of Lesinurad was plotted as per the procedure in experimental method and its linearity is shown in Table 3 and fig. 1. The standard graph of Lesinurad showed good linearity with R^2 of 0.998, which indicates that it obeys "Beer- Lambert's" law in the range 0-28 µg/mL

Table 3: Standard graph of Lesinurad in phosphate buffer pH 6.8

Concentration (µg/mL)	Absorbance
0	0
4	0.121
8	0.233
12	0.388
16	0.503
20	0.606
24	0.717
28	0.859

Table 3 : Physical Properties of Precompression Blend

Formulation Code	Angle of repose (°)	Bulk density (g/mL)	Tapped density(g/mL)	Carr's index (%)	Hausner's ratio
F1	26.76±1.2	0.526±1.8	0.612±1.6	14.0±0.02	1.16±0.1
F2	27.54±2.5	0.662±1.2	0.763±1.3	13.23±0.1	1.15±0.05
F3	24.65±2.5	0.695±1.5	0.823±0.8	15.5±0.08	1.18±0.1
F4	22.9±1.4	0.672±1.2	0.742±1.2	12.2±0.1	1.21±0.2
F5	28.3±2.2	0.643±2.1	0.624±0.7	14.2±0.9	1.11±0.2
F6	24.84±0.4	0.654±1.6	0.755±1.4	13.12±1.8	1.12±0.06
F7	28.68±0.8	0.782±1.2	0.869±0.8	11.0±1.2	1.11±0.2
F8	24.68±1.2	0.560±0.5	0.631±1.2	11.25±0.15	1.12±0.08
F9	25.16±0.8	0.628±2.5	0.714±1.6	14.27±0.12	1.17±0.5

Each value represents the mean ±SD (n=3)

Physicochemical Characterization Of Buccal Tablets

In all the formulations, the weight variation of tablets revealed that the tablets were within the range of pharmacopoeial limit. Hardness test indicated good mechanical strength, the hardness of prepared salbutamol buccal tablets was found to be in the range of 5.2 to 6.0 kg/cm². Thickness of the tablets was ranges from 3.37 to 3.22 mm. The friability of the buccal tablets of all the batches remained in the range of 0.55 to 0.43 %. Friability is less than 1%, indicated that tablets

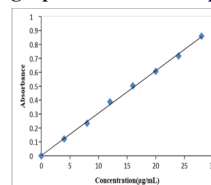
Table 4: Physico-chemical parameters of Lesinurad buccal tablets:

Formulation Code	Average Weight (mg)	Thickness (mm)	Hardness (kg/cm ²)	Friability (%)	Assay (%)
F1	450.74 ± 0.61	3.37 ± 0.03	5.2±0.14	0.55±0.018	99.65 ± 0.44
F2	450.04 ± 0.80	3.34 ± 0.02	5.3±0.29	0.63±0.019	99.13 ± 0.75
F3	450.38 ± 0.71	3.36 ± 0.03	5.2±0.49	0.66±0.022	99.28 ± 0.92
F4	450.45 ± 0.64	3.36 ± 0.02	5.4±0.17	0.58±0.026	98.77 ± 1.00

used. The presence of peaks at the expected range confirms that the materials taken for the study are genuine and there were no possible interactions shown in Table 2.

Solubility studies

Solubility of Lesinurad in the phosphate buffer, pH 6.8 was conducted and it was found to be 89µg/mL. The study was conducted in phosphate buffer, pH 6.8. The flux and permeability coefficient of drug solution was found to be 0.3755 mg h⁻¹ cm⁻² and 0.01545 cm h⁻¹, respectively Saturation solubility of Lesinurad in various buffers were studied and shown in the Table 2

Figure 6: Standard graph of Lesinurad in phosphate buffer pH 6.8**Characterisation of Precompression Blend:**

The precompression blend for Buccal tablets were characterized with respect to angle of repose, bulk density, tapped density, Hausner's ratio, Carr's index and drug content and shown in the Table 26. Angle of repose was less than 30° and Carr's index values were less than 18 for the precompression blend of all the batches indicating good to fair flowability and compressibility, Hausner's ratio was less than 1.25 for all the batches indicating good flow properties. The blend of ingredients was analyzed for physical characteristics. The angle of repose of formulation blends F1 to F9 was found to be in the range of 26.76 to 25.16 ° which indicate excellent, The bulk density, tapped density were found in the range of 0.526 to 0.628gm/cc, 0.612-0.714 gm/cc, Carr's index in the range of 14.0 to 14.2 % and Hausner's ratio in the range of 1.16 to 1.17. It reveals that all the formulation blends were having good flow characteristics and flow rates were shown in Table 3.

had a good mechanical resistance. The evaluation parameters were within acceptable range for all the formulations. The drug content estimation showed values in the range of 98.69±1.00 to 99.81±0.04 which reflects good uniformity in the drug content among different formulations. Assay of all compressed tablets were within the limits as per USP.

The results of content uniformity indicated that the drug uniformly dispersed. The results of weight variation, hardness, thickness, friability and the drug content were shown in Table 4.

F5	449.91 ± 1.01	3.21 ± 0.02	5.5±0.28	0.64±0.021	98.96 ± 0.44
F6	449.98 ± 0.82	3.34± 0.01	5.9±0.24	0.47±0.017	98.81 ± 0.92
F7	450.38 ± 0.80	3.68± 0.02	6.8±0.17	0.66±0.022	99.77 ± 0.72
F8	450.04 ± 0.71	3.34± 0.03	6.5±0.49	0.65±0.021	99.81 ± 0.44
F9	449.94 ± 0.75	3.22± 0.02	6.0±0.19	0.43±0.015	99.15 ± 0.75

Each value represents the mean ±SD (n=3).

In vitro drug release studies:

The Release of lesinurad from buccal tablets varied according to type and ratio of matrix forming polymers. The drug release was governed by amount of matrix forming polymers. The most important factor affecting the rate of release from buccal tablets is the drug and polymer ratio. As increase in the polymer concentration increases the viscosity of the gel as well as the formation of gel layer with longer diffusional path. This could cause a decrease in the effective diffusion co-efficient of drug and therefore reduction in drug release rate. In present study, the results followed this predictable behavior Fig:1. Tablets from F1 and F2, F3 sodium alginate as a secondary polymer showed drug release more than 92.3% in 8 hours. Among the three formulations of this group, F1 showed highest drug release composed of 1:0.5 (drug: Sodium Alginate) ratio. This is probably due to high gelling property of sodium alginate. Tablets from F3, F4, F5 Carbopol 934 as a secondary polymer composed of 1:0.5 (drug:carbopol) ratio, F4 showed a

maximum release of 81.23 respectively in 8 hours. Carbopol 934 is reported to be responsible for the formation of the hydrogen bonds between the mucus and functional groups of the polymer. HPMC was used to provide adhesion and controlled drug release. Tablets of F7 and F8 HPMC K 200Mas a secondary polymer composed of 1:0.75 (drug: HPMC K 200M) ratio showed a maximum release of 98.6% in 8 hours. It is observed that as increase in the polymer concentration, causes an increase in the viscosity of the gel as well as formation of a gel layer with a longer diffusion path and decrease in diffusion coefficient of drug. Therefore, increased in polymers concentration leads to decrease in drug release, there was a reduction in the amount of polymer ensures faster release. This may be attributed due to reduction in strength of gel layer which enhances drug diffusion and water uptake through matrix. Finally it was concluded that, *In vitro* drug release studies revealed that the release of Lesinurad from different formulations varied according to the type and ratios of the matrix forming polymers.

Table 5: In vitro cumulative percentage drug release profile of Lesinurad formulations

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	49.67±0.53	10.965±0.63	21.85±0.79	29.125 ±0.24	7.037±0.43	14.32±0.31	29.11±0.02	35.14±0.52	18.14±0.33
2	53.36±0.35	13.257±0.15	32.15±0.48	42.22 ±0.41	9.24±0.35	20.867±0.50	33.13±0.5	48.03±0.12	23.58±0.78
3	66.05±1.11	22.53±0.44	36.17±0.13	47.12 ±0.55	22.536±0.74	27.9±0.72	37.8±0.53	51.62±0.49	35.425±0.44
4	76.39±0.51	29.87±0.72	42.55±0.59	56.05 ±0.31	29.9±0.45	30.85±0.49	43.05±0.25	58.92±0.51	54.675±0.33
5	80.05±0.62	32.11±0.94	47.62±0.72	61.6 ±0.78	32.11±0.72	34.62±0.65	54.82±0.68	70.06±0.36	83.475±0.55
6	84.3±0.74	38.44±0.45	51.22±1.29	65.7±0.45	38.4±0.57	39.07±0.41	60.88±0.75	82.53±0.64	85.925±0.77
7	90.025±1.18	38.74±0.82	53.85±0.35	72.6±0.11	49.55±0.97	45.98±0.75	62.22±0.46	97.85±0.56	87.75±1.23
8	92.23±0.72	43.09±0.76	58.98±0.65	81.23±0.23	67.77±0.64	58.71±0.39	69.05±0.54	98.32±0.23	89.32±0.97

Each value represents the mean ± SD (n=3)

Surface pH study

The bio-adhesion strength, ex vivo residence time and surface pH of all formulation were given in Table 6. In order to know the irritation potential in vivo, surface pH study was performed because acidic or alkaline pH may cause irritation to the mucosa. Surface pH of the optimized formulation F8 was found to be 7.16 (near to alkaline pH) suggesting that it do not cause any irritation to the mucosa.

Ex vivo residence time

The bioadhesion strength, ex vivo residence time and surface pH of all formulation were given in Table 6. The minimum and maximum ex vivo residence time were found to be 5h 42 min., 7h, 45 min. respectively. The optimized formulation (F8) showed ex vivo residence time of 6h.15min. As the polymer concentration increased, bioadhesion strength and ex vivo residence time increased.

Moisture absorption

The moisture absorption studies of tablets give an indication of the relative moisture absorption capacities of polymers and whether the formulations would maintain their integrity after moisture absorption. The order of increasing moisture absorption was Sodium alginate < Carbopol 934 < HPMC K 200M (Table 6). The higher moisture absorption of HPMC K200M may be due its predominant hydrophilic nature.

Mathematical model fitting of in vitro drug release

Ex vivo residence time, Moisture absorption, Surface pH, Bioadhesive strength values of Lesinurad buccal tablets

Formulation code	Ex vivo residence time	Moisture absorbance	Surface pH	Bioadhesive strength	
				Peak detachment force (N)	Work of adhesion (mJ)
F1	5Hrs 42 min	30.83± 0.25	6.96±0.16	1.89±0.55	0.47±0.28
F2	5 Hrs 15 min	25.66 ± 0.25	6.86±0.43	2.34±0.02	0.62±0.04
F3	5Hrs 45 min	32.45 ± 0.25	6.9±0.35	2.05±0.42	0.5±0.28
F4	5 Hrs 35 min	17.51 ± 0.30	6.5±0.12	1.24±0.38	0.34±0.17
F5	5 Hrs 20 min	9.61±0.25	6.66±0.23	2.42±0.06	0.74±0.02
F6	5 Hrs 15 min	20.83±0.25	7.43±0.15	1.30±0.12	0.40±0.38
F7	6Hrs 10 min	14.16±0.25	6.8±0.43	2.30±0.26	0.61±0.13
F8	6 Hrs 15 min	13.33±0.30	7.16±0.24	2.68±0.03	0.95±0.08
F9	7Hrs 45 min	19.16±0.30	6.67±0.13	2.44±0.47	0.69±0.41

The in vitro percentage drug release of optimized formulation F3 was attempted to fit into mathematical models. The n and R2 values for zero-order, first-order and Higuchi and Peppas were represented in Table 7. The Peppas model is widely used, when the release mechanism is not well known and when more than one type of release is involved (Peppas,1985). The semiempirical equation is shown as Eq. 3:

$$M_t/M_\infty = k t^n$$

Where M_t/M_∞ is fraction of the drug released at time t; k represents a constant, incorporating structural and geometrical characteristics of the buccal devices; and n is the diffusion exponent, which characterizes the type of release mechanism during the dissolution process. For non-Fickian release, the value of n falls between 0.5 and 1.0, while in case of Fickian diffusion, n=0.5; for zero order release (case II transport), n=1; and for supercase II transport, n is greater than 1. Observation of all the R2 values indicated that the highest R2 (0.9988) value was found for Zero order release. According to 'n' value it is less than 0.5, so it follows fickian diffusion with zero order release were shown in table 7.

Ex vivo bioadhesive strength measurement:

The values of the bioadhesive strength of Lesinurad buccal tablets of different formulations were given in Table 8. The bioadhesive characters were found to be affected by the nature and proportions of the bioadhesive polymers used in the formulations. Hence the formulation with optimized bioadhesive strength should be chosen i.e. formulation containing HPMC K 200M.

Each value represents the mean $\pm$ SD (n=3)

Swelling Studies of buccal tablets

Appropriate swelling property of a buccal device is essential for uniform and prolonged release of drug and proper bioadhesion. The polymeric tablet formulations displayed an increase in weight due to water uptake. The mucoadhesive polymers (sodium alginate gum, Carbopol 940 and HPMC K 200M) used in this study were hydrogels which are swellable upon contact with water and retain large amount of water. From the results, the highest percentage swelling is to the formulation F8 i.e., 143.7% at the 8 hours. Swelling behavior of buccal tablets of all formulations as a function of time is shown in Fig.7

Table 32: Swelling studies of buccal tablets

Time (hr)	% Swelling index								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	19.6	28	4.0	11.2	2.4	3.8	33.8	14.9	18.8
2	39.6	75.4	10.7	18.6	12.2	14.4	77.5	39.3	26.3
3	80.4	84.7	44.0	46.0	15.8	17.9	85.5	62.0	58.9
4	132	114.4	99.8	46.8	35.4	36.4	126	102.9	102.5
5	175.6	155.9	148.1	71.7	37.8	42.3	130.7	137.5	123.4
6	197.5	212.4	197.3	91.2	41.5	49.7	142.8	143.7	135.7

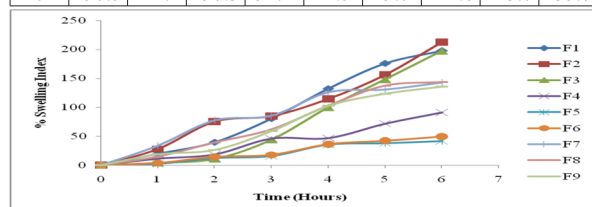


Figure 7: Swelling index of Lesinurad buccal tablets

ACCELERATED STABILITY STUDIES :

The stability study of the optimised tablets were carried out according to ICH guidelines at $40 \pm 2^\circ\text{C}/75 \pm 5\%$ RH for three months by storing the samples in (Lab-care, Mumbai) stability chamber. The results from stability studies are shown in table 9.

Table 11: Release kinetics and correlation coefficients

Time (T)	Root (T)	Log(%) Release	Log (T)	Log (%) Remain	Release Rate (cumulative % Release / T)	1/CUM% Release	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0			2.000				100	4.642	4.642	0.000
1	35.14	1.270	0.000	1.911	18.600	0.0538	-0.730	81.4	4.642	4.334	0.308
2	48.03	1.433	0.301	1.863	13.550	0.0369	-0.567	72.9	4.642	4.177	0.464
3	51.62	1.535	0.477	1.818	11.433	0.0292	-0.465	65.7	4.642	4.035	0.606
4	58.92	1.668	0.602	1.728	11.650	0.0215	-0.332	53.4	4.642	3.766	0.876
5	70.06	1.713	0.699	1.685	10.320	0.0194	-0.287	48.4	4.642	3.644	0.997
6	82.53	1.777	0.778	1.604	9.967	0.0167	-0.223	40.2	4.642	3.426	1.216
7	97.85	1.822	0.845	1.526	9.486	0.0151	-0.178	33.6	4.642	3.227	1.415
8	98.32	1.862	0.903	1.435	9.100	0.0137	-0.138	27.2	4.642	3.007	1.634

Figure 8 : Zero order release kinetics

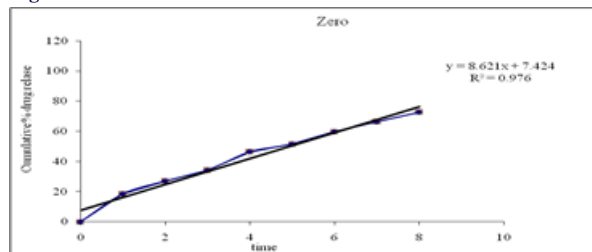


Figure 9 : Higuchi release kinetics

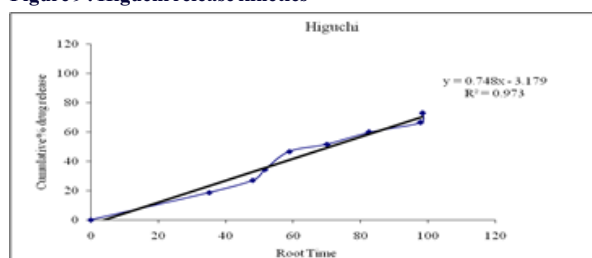


Table No. 9 : Stability dissolution profile of F8 for 1st, 2nd & 3rd months

S. No	Time (hours)	F8 (1 st Month)	F8 (2 nd Month)	F8 (3 rd Month)
1	0	0	0	0
2	1	35.14 $\pm$ 0.52	34.34 $\pm$ 0.61	34.24 $\pm$ 0.58
3	2	48.03 $\pm$ 0.12	49.24 $\pm$ 0.13	48.03 $\pm$ 0.17
4	3	51.62 $\pm$ 0.49	50.12 $\pm$ 0.53	50.11 $\pm$ 0.43
5	4	58.92 $\pm$ 0.51	57.73 $\pm$ 0.55	57.99 $\pm$ 0.50
6	5	70.06 $\pm$ 0.36	70.19 $\pm$ 0.45	71.08 $\pm$ 0.36
7	6	82.53 $\pm$ 0.64	83.74 $\pm$ 0.91	82.52 $\pm$ 0.61
8	7	97.85 $\pm$ 0.56	97.54 $\pm$ 0.78	96.71 $\pm$ 0.45
9	8	98.32 $\pm$ 0.23	98.31 $\pm$ 0.22	98.33 $\pm$ 0.38

Table 10 : Physicochemical parameters of most satisfactory formulation during stability studies for optimised formulation

Time Period (Month)	Hardness (kg/cm ²)	Drug Content (%)
1	6.4 $\pm$ 0.48	99.81 $\pm$ 0.40
2	6.5 $\pm$ 0.47	99.80 $\pm$ 0.43
3	6.4 $\pm$ 0.48	99.80 $\pm$ 0.42

There was no major change in the various physicochemical parameters evaluated like hardness, drug content, *in vitro* dissolution pattern at the various sampling points. There was no statistically significant difference between the initial values and the results obtained during stability studies.

Kinetic Study:

To analyze the mechanism of drug release from the tablets the *in vitro* dissolution data were fitted to zero order ($K=kt$), korsmeyer and peppas model ($F=ktn$), higuchi ($F=kt$) release models. Where F is the fraction of drug release, k is the release constant and t is time. The details are given in table 11. The optimized formulation such as HPMC K 200 M (F8) follows First order release kinetics governed by Fickian diffusion mechanism. Formulation F8 was considered to be the optimized one based on the result of *ex vivo* bioadhesive strength, mucoadhesion time and *in-vitro* drug release, hence it was chosen for carrying out the *in-vitro* permeation studies across Sheep buccal mucosa. The drug penetration was slow and uniform could permeate through the buccal mucosa in 8 hrs with a flux of 0.0499 mg/hr/cm²

Figure 10 : Kors mayer peppas release kinetics

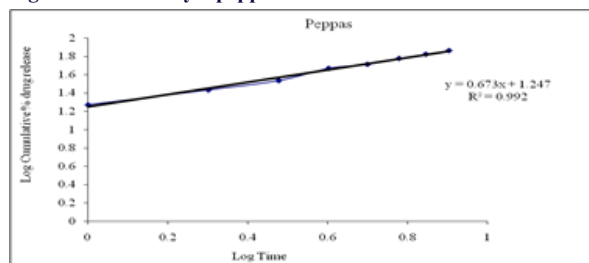
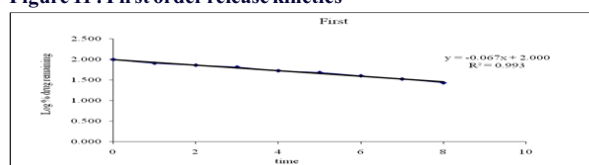


Figure 11 : First order release kinetics



CONCLUSION:

Development of bioadhesive buccal drug delivery lesinurad bioadhesive buccal tablets was one of the alternative routes of

administration to avoid first-pass effect, to improve the bioavailability of lesinurad through buccal mucosa and provide prolonged release. In addition, these formulations reduce the need of frequent administration and enhance patient compliance. The *in vitro* drug release was found to be Fickian and kinetics drug release result reveals that all formulations follow first-order kinetics. The formulated unidirectional buccoadhesive tablets using HPMC as mucoadhesive agent is superior to oral conventional tablets, as it has the potential to bypass the first pass metabolism and improve the bioavailability of lesinurad. It is therefore expected to reduce adverse effect, cost and ultimately improve the patient compliance.

ACKNOWLEDGEMENT: The authors are thankful to the management of Guru Nanak Institute of Pharmaceutical Sciences for their sheer support throughout the work. The authors also express their thanks to Sura Labs, Hyderabad for their extensive support in conducting FTIR, Stability studies.

CONFLICTS OF INTEREST: The authors have no conflicts of interest to declare.

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