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# Development, *In vitro*, *Ex vivo* and *In vivo* Evaluation of Mucoadhesive Buccal Tablets of Hydralazine Hydrochloride

SK Wajid[1], Ajmeera Ramarao*[2]

[1] Department of Pharmacy, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan, 333001 (India)
[2] Department of Pharmaceutical Analysis, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, 5060093 (India)

* For correspondence
Email: ramaraoajmera@gmail.com

**Key words**
Hydralazine hydrochloride, First-pass metabolism, Buccal mucoadhesive tablets

**Abstract**
Hydralazine hydrochloride is an anti-hypertensive drug. The drug has poor oral bioavailability (BA) of about 30- 50% due to extensive first-pass metabolism. Hence, the buccal tablets were used to enhance the BA of hydralazine hydrochloride. Buccal muco-adhesive tablets were prepared by direct compression technique, using carbopol 934P and HPMC K4M as muco-adhesive polymers. Prepared formulations were evaluated for physico-chemical characterization, *ex vivo* residence time and *in vitro* release studies. Further, *in vivo* studies conducted in pigs comparison with marketed formulation the some of the parameters viz hardness, thickness, weight variation are showing the values within the pharmacopeial limits. However, the swelling and bio-adhesive strength were increased with increasing polymer concentrations. From the *in vitro* release studies it is shown that, tablets with HPMC K4M (F2) exhibited better release profile than all other formulation, which may be due to the hydrophilicity and water uptake by the tablet and were considered as optimized formulation. The release mechanism from kinetic methods suggests that, the drug release follows zero-order kinetics with diffusion mechanism. Further, about 3.09-folds enhancement in the oral bioavailability of HH from buccal tablet was observed when compared with immediate release marketed formulation. Thus, the buccal tablets of hydralazine hydrochloride showed enhanced BA and were further confirmed by *in vivo* studies.

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## INTRODUCTION

Oral route is most preferred and widely applicable route for the delivery of majority of the drugs. But the problems such as poor aqueous solubility, less residence time, chemical instability in the gastrointestinal tract minimizes the bioavailability (BA) of orally administered drugs.[1] Further, metabolism through various barriers or enzymes also degrade the drug before reaching site of action. Hence, various alternative drug delivery systems are developed to enhance the oral BA of these drugs. The delivery systems include; enhancement of solubility through solid dispersions[2], complexation with cyclodextrins[3], liquisolid compacts[4]; increase the stability and prolonged residence time through floating systems[5,6], increase the mucoadhesive property[7]; lipid based delivery systems for by passing metabolism with solid lipid nano particles[8], transfersomes[9], nanostructured lipid carriers and micronization for reducing particle size using nanosuspensions.[10,11]

The oral cavity is easily accessible for self-medication and is well accepted by patients. In the last three decades, there is a great interest in the research of buccal drug delivery system.[12] The oral cavity is the most attractive route for drug delivery due to its ease of administration. Both locally acting and systemic acting drugs can be administered by this route. The site-specific release of drug at mucosa is achieved when used for local activity and systemic action requires drug absorption through the mucosal barrier to reach systemic circulation.[13] In the last three decades, there is a great interest in the research of buccal drug delivery system. Buccal delivery of drugs provides an attractive alternate to the oral route of drug administration, particularly in overcoming deficiencies associated with the oral route. Drug delivery via the buccal route using bioadhesive dosage forms offers a novel route of drug administration.[14,15,16]

Hydralazine hydrochloride (HH) is used widely for treating hypertension. The drug is well absorbed from the gastrointestinal tract but its bioavailability is low (30- 50%) due to extensive first pass metabolism. Since the buccal route bypasses the first-pass effect, the dose of hydralazine hydrochloride could be reduced by 50%. The physicochemical properties of hydralazine hydrochloride, its suitable half-life (3-7 h) and low molecular weight (196.64) and used the oral route for small dose and absence of objectionable taste and odor make it a suitable candidate for buccal administration.

The aim of the present research was to develop and evaluate the mucoadhesive buccal tablets of HH to improve the oral bioavailability.

## EXPERIMENTAL

### Materials

Hydralazine hydrochloride was obtained as a gift sample from Stride's lab, Bangalore India. Carbopol 934P was obtained from S.D. Fine Chemicals, Mumbai. Hydroxy propyl methyl cellulose (HPMC K4M) and carbopol was obtained from Loba chemicals, Mumbai. Micro crystalline cellulose (MCC) obtained from Laksmi chemicals, India PEG 6000 obtained from India glycol Pvt Ltd., Mumbai, India. All other ingredients used in formulations were of analytical grade.

### Drug-Polymer Compatibility using DSC

The DSC is a useful tool for determining the drug excipient interaction for the development formulation. The thermal properties of the drug and the mixture of drug and excipients are of important interest since this can help to assess the interaction among different components of the formulations. The DSC of pure drug, polymer, physical mixture of drug-polymer and optimized formulation were determined. For DSC study, Universal V4 TA instruments was used, samples 2–4 mg was weighed accurately, placed in aluminum pans and heated at 10°C per min rate in the range of 30-300°C in a nitrogen purging gas environment

### Preparation of HH Buccal Adhesive tablets (HH-BT)

Buccal tablets of HH (HH-BT) were prepared by a direct compression method, before going to direct compression all the ingredients were screened through sieve no.100. Drug was mixed manually with different ratios of mucoadhesive polymers and diluent for 10 min. The blend was mixed with magnesium stearate for 3-5 min and then compressed into tablets by the direct compression method using 6 mm flat faced punches. The tablets were compressed using a sixteen station Cemach rotary tablet-punching

machine. The mass of the tablets was determined using a digital balance (Shimadzu) and thickness with digital screw gauge. The composition is depicted in Table 1. Before compression, the blends were characterized with respect to angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio.

Table 1. Formulation of mucoadhesive buccal tablets of hydralazine hydrochloride

| Ingredients (mg) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| **Hydralazine Hydrochloride** | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| **HPMC $K_{15}M$** | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 25 |
| **Carbopol 934 P** | - | - | - | - | 25 | 25 | 25 | 75 |
| **Microcrystalline cellulose** | 110 | 85 | 60 | 35 | 85 | 60 | 35 | 35 |
| **PVP k30** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Magnessium Stearate** | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| **Talc** | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| **Total weight (mg)** | 175 | 175 | 175 | 175 | 175 | 175 | 175 | 175 |

## Evaluation of Mucoadhesive Buccal Tablets

### *Determination of weight variation*

This is an important quality control test to be checked for any variation in the weight of tablets that leads to either under or overdose. So, every batch should have a uniform weight. Twenty tablets were randomly selected from each formulation and their average weight and standard deviation were calculated from the total weight of all tablets. The % difference in the weight variation should be within the permissible limits. The % deviation was calculated.

### *Thickness*

The thickness of buccal tablets was determined with the help of Vernier calipers. Three individual tablets from each formulation were used and the results averaged.

### *Hardness*

Hardness is an important quality control test to be indicated for measuring the ability of a tablet to withstand mechanical shocks while handling. The test was conducted for 3 tablets from each formulation using Monsanto hardness tester; the average and standard deviation values were calculated.

### *Friability*

It is a measure of mechanical strength of tablets. Roche friabilator was used to determine the friability by the following procedure. Pre-weighed tablets (10 tablets) were placed in the friabilator. This device consists of a plastic chamber that was set to revolve around 100 rpm for 4 minutes dropping the tablets at a distance of 6 inches with each revolution. At the end of the test, tablets were reweighed; loss in the weight of the tablet is the measure of friability and is expressed in percentage as:

$$F\ (\%) = [1\text{-}W_F/Wo] \times 100 \quad (1)$$

Where, Wo is the weight of the tablets before the test and $W_F$ is the weight of the tablets after test.

### *Drug content*

Ten tablets were weighed and grounded in a mortar with a pestle to get fine powder; powder equivalent to the mass of one tablet was dissolved in phosphate buffer pH 6.8 for 10 min, sufficient buffer was added

and filtered; 1 mL of filtrate was suitably diluted with buffer and drug content was analyzed spectrophotometrically at 260 nm using a UV spectrophotometer.

### Swelling studies

Appropriate swelling behavior of a buccal adhesive system is an essential property for uniform and effective muco-adhesion. Swelling of tablet involves the absorption of a liquid resulting in an increase in weight and volume. The swelling behavior of a buccal adhesive system is an important property for uniform and prolonged release of drug and bio-adhesiveness. The agar plate model used in this study resembles the secreting fluid around the buccal mucosa. For each formulation, three buccal tablets were weighed individually ($W_1$) and placed separately in 2% agar gel plates with the core facing the gel surface and incubated at 37 ± 1°C. After every 1 h time interval until 6 h, the tablet was removed from the petri-dish and excess surface water was removed carefully with blotting paper. The swollen tablet was then reweighed ($W_2$) and the swelling index (SI) was calculated using the following formula:[13]

$$\text{Swelling Index} = [(W_2-W_1) \div W_1] \times 100 \quad (2)$$

Where, $W_1$ is initial weight of the tablet and $W_2$ is final weight of the swollen tablet.

### Surface pH

The bioadhesive tablets were allowed to swell by keeping it in contact with 1 mL of distilled water for 2 h at room temperature. Thereafter, surface pH measurements at predetermined intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h were recorded with the aid of a digital pH meter. These measurements were conducted by bringing a pH electrode near the surface of the tablets and allowing it to equilibrate for 1 min prior to recording the readings. Experiments were performed in triplicate.

### Moisture absorption

Agar (5% m/V) was dissolved in hot water. It was 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 by using following formula:

$$\text{Moisture Absorption (\%)} = [(\text{Final weight} - \text{Initial weight}) / \text{Initial weight}] \times 100 \quad (3)$$

### Ex vivo drug permeation

*In vitro* permeation study of HH buccal tablets through the porcine buccal mucosa was performed using Franz-type diffusion cell with a diffusion area of 30.02 $cm^2$ and the receptor compartment volume of 21 mL at 37 ± 0.2°C and 50 rpm. This temperature and rpm was maintained by using magnetic stirrer. Porcine buccal mucosa was obtained from a local slaughter house and used within 2 h of slaughter. The tissue was stored in Krebs buffer at 4°C upon collection. The epithelium was separated from underlying connective tissues with surgical scissors and clamped between donor and receiver chambers of the Franz-type diffusion cell. After the buccal membrane was equilibrated for 30 min with Krebs buffer solution between both the chambers, the receiver chamber was filled with fresh pH 7.4 buffer solution.

The buccal tablet was placed in donor chamber and wetted with 1 mL of buffer solution (pH 6.8).[3] The amount of drug permeated through the membrane was determined by removing aliquots (5 mL) were collected from the receiver chamber at predetermined time intervals and filtered through a filter paper and the medium of the same volume (5 mL), which was pre-warmed at 37°C, was then replaced into the receiver chamber. The amount of drug permeated through the buccal mucosa was then determined by measuring the absorbance at 236 nm using a UV spectrophotometer. The experiments were performed in triplicate and mean value was used to calculate the flux (J) and permeability coefficient (P).

$$J = (dQ/dt) / A \quad (4)$$

$$P = (dQ/dt) / \Delta CA \quad (5)$$

Where, J is the steady-state flux ($mg.hrs^{-1}cm^{-2}$), P is permeability coefficient (cm/h), dQ/dt is the slope obtained from the steady state portion of the curve, ΔC is the concentration difference across the mucosa and A - the area of diffusion ($cm^2$).

### Kinetics of drug release and mechanism

To know the release mechanism and kinetics of Hydralazine hydrochloride, the release data was fitted into mathematical models and n, R2 values for zero order, First order, Higuchi and Peppas' models were calculated.

### Ex vivo residence time

The *Ex vivo* residence time is one of the important physical parameter of buccal mucoadhesive tablet. The adhesive tablet was pressed over excised pig mucosa for 30 sec after previously being secured on glass slab and was immersed in a basket of the dissolution apparatus containing around 500 mL of phosphate buffer, pH 6.8, at 37°C. The paddle of the dissolution apparatus as adjusted at a distance of 5 cm from the tablet and rotated at 25 rpm. The time for complete erosion or detachment from the mucosa was recorded.

### Ex vivo bioadhesion strength

Bioadhesion strength of tablets were evaluated using a microprocessor based on advanced force guaze equipped with a motorized test stand (Ultra Test Tensile strength tester, Mecmesin, UK) according to method described as it is fitted with 25 kg 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 (1 %w/v, 100 µL) 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 0.5 mm/sec until the tablet was completely detached from the mucosa. The work of adhesion was determined from the area under the force distance curve. The peak detachment force was maximum force to detach the tablet from the mucosa.

$$\text{Force of adhesion} = [\text{Bioadhesion strength} \times 9.8] / 1000 \quad (6)$$

$$\text{Bond strength} = \text{Force of adhesion} / \text{Surface area} \quad (7)$$

### Stability studies

Stability studies were performed for a period of 90 days for the optimized buccal tablet. Sufficient number of tablets (15) was packed in amber colored screw capped bottle sand kept in stability chamber maintained at 40 ± 1°C and 75% RH. Samples were taken at monthly intervals for drug content estimation. At the end of three months' period, dissolution test and drug content studies were performed to determine the drug release profiles and drug content.

### Comparative bioavailability in pigs

The study protocol was reviewed and approved by the creature moral advisory group, Browns College of Pharmacy, Khammam, Telangana (CPCSEA Approval No: 1641/PO/E/S/14/CPCSEA). White healthy pigs weighing 30 ± 5 kg were selected from the slaughter house for the study. The bioavailability of optimized mucoadhesive buccal tablet (HH-BT) was compared with marketed tablet formulation (Nepresol ®). The animals were allowed free access to food and water, until the night prior to dosing, and were fasted for 10 h. Latin square cross-over design was followed; the animals were divided into two groups, each group consisting of six pigs. To one group, marketed formulation was administered through feeding tube followed by rinsing with 10 mL of water and HH-BT to another group in the first phase. The pigs were anesthetized during sample collection until the third hour sample. In the second phase vice versa was followed and was conducted after 15 days of wash out period. Blood samples (5 mL) from the tail vein were collected at pre-set time intervals. All blood samples were allowed to clot and centrifuged for 10 min at 5000 rpm (MIKRO 220R, Hettich, Germany). The serum was separated and transferred into clean micro-centrifuge tubes and stored at −20°C until HPLC analysis of the drug.

### *Pharmacokinetics*

Pharmacokinetic parameters of HH after administration of bioadhesive buccal and marketed tablet formulations were estimated for each animal using a computer program, KINETICA 2000 (Version 3.0, Innaphase Corporation, PA, USA). Non-compartmental analysis was used to calculate the pharmacokinetic parameters: mean peak plasma concentration ($C_{max}$), time to reach peak plasma concentration ($T_{max}$), and area under the curve (AUC). The relative bioavailability (F) for buccal delivery was calculated using equation below:

Relative Bioavailability = AUC of buccal tablet / AUC of marketed tablet (8)

## RESULTS AND DISCUSSION

### *Compatibility studies*

The DSC thermogram of pure hydralazine hydrochloride showed endothermic peak at a temperature of 164.7°C, which is corresponding to its melting range (163.1 – 168.9°C). The pure HPMC K4M polymer showed decomposed endothermic peak at 76.88°C. In case of physical mixture, the drug showed an endothermic peak at 165.3°C and polymer at 75.77°C. The optimized formulation showed drug peak at 157.7°C and HPMC K4M polymer at 71.77°C, respectively (Figure 1). From this observation, there was no interaction takes place between drug and polymer.

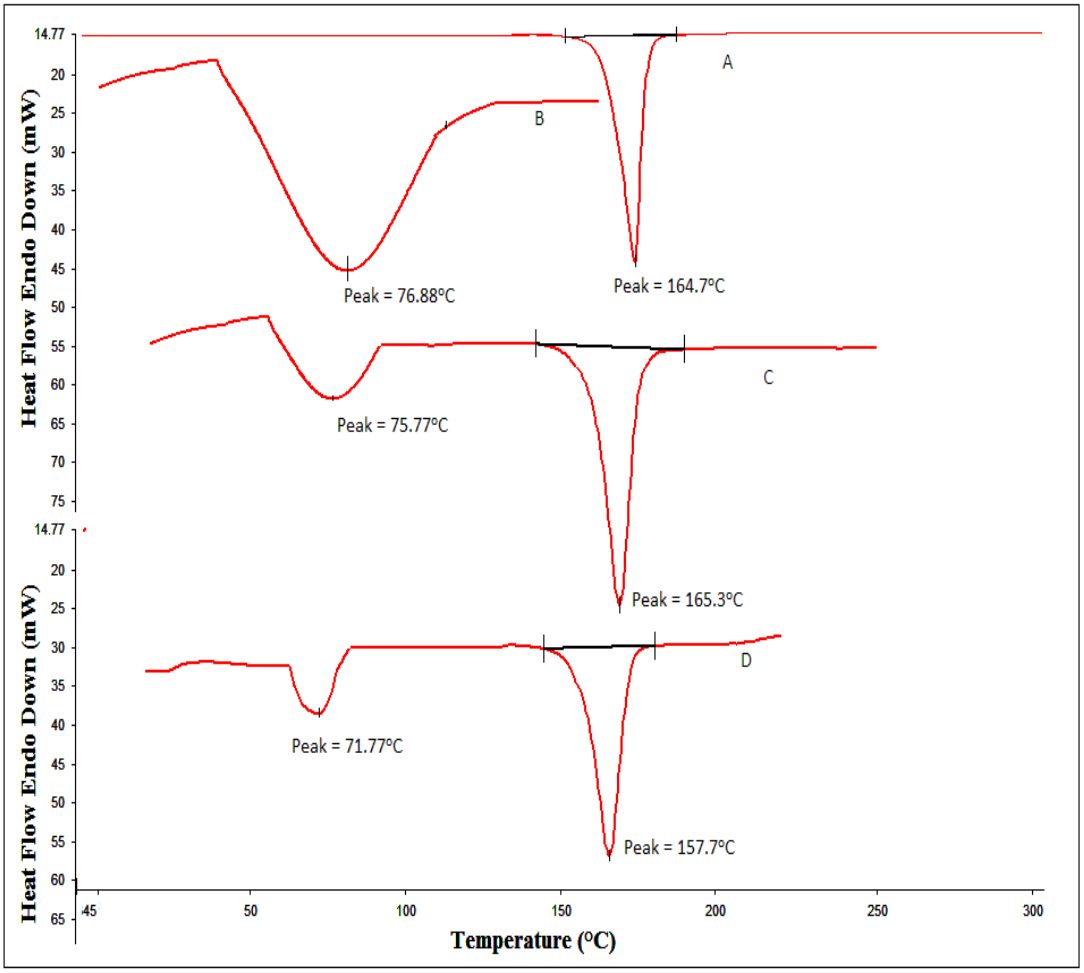


Figure 1. DSC thermograms of pure drug (A), pure HPMC K4M (B), physical mixture of drug and HPMC K4M (1:1) (C) and optimized formulation (F2) (D)

### Characterization of blend

The Angle of repose was less than 30° and Carr's index values were less than 15 for the blend of all the batches indicating excellent to good flowability and compressibility. Hausner's ratio was less than 1.11 for all the batches indicating excellent flow properties. The results are represented in Table 2.

Table 2. Physical Properties of Pre-Compression blend

| Formulation Code | Angle of repose (θ) | Bulk density(g/cm³) | Tapped density(g/cm³) | Carr's Index (%) | Hausner's Ratio |
|---|---|---|---|---|---|
| **F1** | 36.82± 0.19 | 0.388 ± 1.6 | 0.426 ± 1.2 | 16.22 ± 1.4 | 1.21 ± 0.08 |
| **F2** | 38.35± 0.14 | 0.4222 ± 0.5 | 0.474 ± 1.8 | 19.8 ± 1.8 | 1.24 ± 0.05 |
| **F3** | 39.92± 0.24 | 0.416 ± 0.9 | 0.456 ± 1.4 | 17.22 ± 2.5 | 1.19 ± 0.6 |
| **F4** | 37.10± 0.23 | 0.462 ± 1.2 | 0.521 ± 1.3 | 20.60 ± 1.5 | 1.23 ± 1.8 |
| **F5** | 36.08± 0.21 | 0.503 ± 2.0 | 0.572 ± 2.0 | 18.12 ± 2.5 | 1.2 ± 1.8 |
| **F6** | 38.76± 0.15 | 0.483 ± 0.9 | 0.592 ± 1.2 | 19.34 ± 2.0 | 1.24 ± 0.4 |
| **F7** | 37.12± 0.14 | 0.519 ± 1.6 | 0.534 ± 0.9 | 20.2 ± 1.8 | 1.25 ± 0.8 |
| **F8** | 39.29± 0.14 | 0.524 ± 1.4 | 0.611 ± 1.2 | 17.58 ± 2.4 | 1.22 ± 0.6 |

### Physicochemical properties of buccal tablets

The values of weight variation and thickness of the tablets (Table 3) were found to be within the limits of conventional oral tablets stated in the Indian Pharmacopoeia. The mass ranged from 172 to 178 mg. Thickness of the tablets varied from 2.22 mm to 2.34 mm. Hardness of the tablets was in the range 3 to 3.5 kg/cm². The mass, thickness and hardness of all compressed tablets were within the limits as per USP.

The drug content ranged from 95.44 to 102.46.The friability ranged from 0.21 to 0.53. Friability and assay of all compressed tablets were within the limits as per USP (Table 3).

Table 3. Physicochemical properties of HH buccal tablets

| Formulation code | Weight variation (mg) | Thickness (mm) | Hardness (kg/cm²) | Friability (%) | Drug Content (%) |
|---|---|---|---|---|---|
| **F1** | 173 | 2.25.±0.16 | 3.0±0.5 | 0.21 | 95.44 |
| **F2** | 177 | 2.22.±0.13 | 3.3±0.3 | 0.45 | 96.84 |
| **F3** | 175 | 2.28.±0.14 | 3.1±0.5 | 0.53 | 94.56 |
| **F4** | 174 | 2.26.±0.16 | 3.5±0.2 | 0.24 | 97.22 |
| **F5** | 172 | 2.27.±0.15 | 3.3±0.5 | 0.31 | 98.26 |
| **F6** | 174 | 2.24.±0.25 | 3.5±0.2 | 0.37 | 97.33 |
| **F7** | 178 | 2.30.±0.14 | 3.1±0.5 | 0.42 | 102.46 |
| **F8** | 176 | 2.34.±0.17 | 3.2±0.3 | 0.29 | 97.47 |

### Swelling studies

The bioadhesion and drug release profile are dependent upon swelling behavior of the tablets. Swelling index increased as the weight gain by the tablets increased proportionally with the rate of hydration. In swelling study, it was found that the amount of HPMC $K_{15}M$ plays an important role in swelling of the matrix and leads to the drug diffusion. It was observed that swelling rate increased with an increase in Polymer content of the prepared tablets. All the formulations showed good swelling index in the range of 11 to 71%. Formulation F6 containing carbopol had shown maximum swelling index. The results are showed in Table 4.

### Surface pH

The surface pH of all formulations was within a range of 6.2 to 7.0, close to neutral pH. These results reveal that all the formulations provide an acceptable pH in the range of salivary pH (6.6 to 7.0). They did not produce any local irritation to the mucosal route.

Table 4. Percentage Swelling Index of HH buccal tablets (mean ± SD; n=3)

| Formulation Code | Percentage Swelling index of tablets after | | | | | |
|---|---|---|---|---|---|---|
| | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| **F1** | 16.01±0.098 | 25.71±1.10 | 37±0.74 | 46.71±1.10 | 54±0.49 | 62±0.78 |
| **F2** | 19.12±0.084 | 28.04±1.51 | 39±1.03 | 47.9±1.99 | 58±1.57 | 69±2.12 |
| **F3** | 14.98±1.01 | 23.14±1.33 | 32±0.91 | 41.9±1.33 | 53±1.83 | 60.5±1.12 |
| **F4** | 11.14±0.088 | 21.96±0.052 | 29±1.07 | 38.5±1.01 | 49±1.81 | 57.4±1.23 |
| **F5** | 15.36±0.99 | 24.16±1.05 | 31±1.70 | 40.4±1.21 | 51±1.03 | 59.6±1.34 |
| **F6** | 21.31±0.65 | 29.53±0.78 | 38±1.57 | 49.4±1.57 | 59±1.21 | 71.3±0.95 |
| **F7** | 17.61±0.95 | 28.96±1.01 | 37±1.35 | 45.0±0.58 | 55±1.53 | 63.2±1.04 |
| **F8** | 18.66±1.16 | 29.56±1.47 | 36±1.02 | 44.8±1.99 | 52±1.73 | 59.5±2.01 |

### *Moisture absorption*

Moisture absorption studies evaluated the integrity of the formulation upon exposure to moisture. Formulations F2 were eroded in 2 hours with 61.54% w/w. When the tablets were positioned without the backing membrane complete swelling followed by erosion was observed, indicating that the drug release mechanism involves swelling of the polymer initially followed by drug release from the swollen matrix by diffusion.

### *Ex vivo drug permeation study*

The results of percentage drug release are shown in Figures 2 and 3. As the concentration of polymer increased, the drug release decreased. *In vitro* drug release studies revealed that release of Hydralazine hydrochloride from different formulations varies with characteristics and composition of matrix forming polymers as shown in graphs.

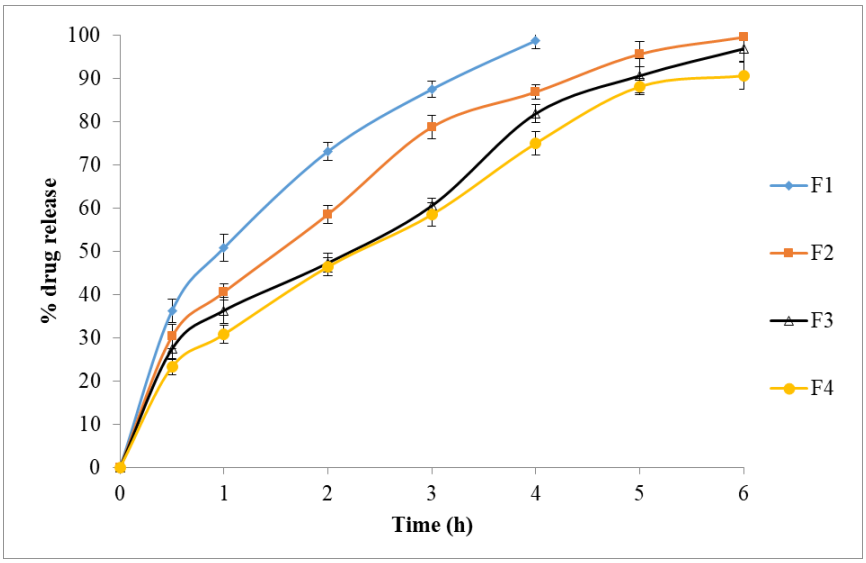


Figure 2. *In vitro* release profiles of buccal tablet formulations from F1 to F4 (mean ± SD, n=3)

An ideal controlled release system should be able to release the drug immediately to attain the therapeutic level at a faster rate and maintain this drug level for a prolonged period of time. The release rate of Hydralazine hydrochloride decreased with increasing concentration of HPMC K4M from F1 to F4 and release rate decreased with increasing the concentration carbopol from F5 to F8. The most important factor affecting the rate of release from the buccal tablets is the drug: polymer ratio. An increase in polymer concentration causes an increase in the viscosity of the gel as well as formation of a gel layer with a longer diffusion path. Increasing the concentration of polymer in the formulations showed a sustained effect on Hydralazine hydrochloride release. The rapidly hydrating polymer dominated in controlling the release of

Hydralazine hydrochloride from the buccal tablets, as seen from the dissolution profiles and moisture absorption data.

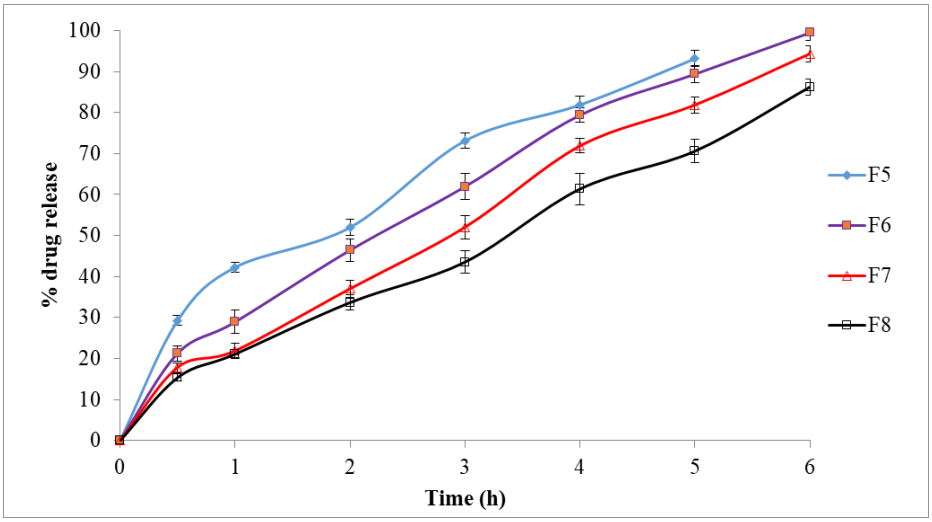


Figure 3. *In vitro* release profiles of buccal tablet formulations from F5 to F8 (mean ± SD, n=3)

### *Kinetics of drug release and mechanism*

To know the release mechanism and kinetics of Hydralazine hydrochloride, the release data was fitted into mathematical models and n, $R^2$ values for zero order, First order, Higuchi and Peppas' models were calculated and are represented in Table 5.

Table 5. *In vitro* release kinetics of the optimized formulation

| Formulation Code | Zero-order | First-order | Higuchi's | Hixon Crowell | Peppas' 'n' value |
|---|---|---|---|---|---|
| **F2** | 0.907 | 0.941 | 0.994 | 0.898 | 0.506 |
| **F6** | 0.976 | 0.836 | 0.980 | 0.819 | 0.648 |

The release exponent '**n**' values were greater than 0.5, which indicates that the drug release from all the batches followed non-Fickian mechanism. The higher R2 values for zero order and Higuchi suggest that the drug release follows zero order kinetics with diffusion mechanism.

Initially, the permeation of HH solution was subjected to diffusion studies. From the results, the cumulative percentage amount of HH permeated in 8 h was found to be 89.88%, while the flux and permeability coefficient of HH were calculated to be 0.668 mg $h^{-1}$ $cm^{-2}$ and 0.056 cm $h^{-1}$, respectively.[17]

### *Ex vivo residence time*

The *ex vivo* residence time is one of the important physical parameter of buccal mucoadhesive tablets. The *ex vivo* mucoadhesive properties of the tablets were determined using porcine buccal mucosa. Formulation F1 to F4 showed lower retention time when compared to the formulation F5 to F8. As the concentration of polymer increased, the retention time increased. This test reflects the adhesive capacity of polymers used in formulations. Optimized formulations i.e., F2 and F6 showed maximum retention time than other formulations.

The oral mucosa of pigs resembles that of humans more closely than any other animal in terms of structure and composition and therefore porcine buccal mucosa was selected for drug permeation studies. The drug permeation was slow and HH could permeate through the buccal membrane with and without enhancer

(PEG 6000) in 8 h. From the *ex vivo* permeation studies, F2 formulation was considered as optimized and used for further studies.

### Ex vivo bioadhesion strength

*Ex vivo* bioadhesion for optimized HH buccal tablets was conducted and displayed good bioadhesion i.e., work of adhesion 3.88 ±0.52 mJ, peak detachment force 1.93±0.09 N.

### Stability studies

Results from stability studies indicate that the formulated HH mucoadhesive tablets are stable for a period of 3 months under 2 different conditions at 25±2°C / 65±5% RH and 40±2°C and 75±5% RH. There were no remarkable changes were observed during the period of storage (Table 6).

Table 6. Stability studies of HH mucoadhesive tablet (F2) at room temperature (mean±SD, n=3)

| Time | Assay | | Cumulative % drug release | | Surface pH | |
|---|---|---|---|---|---|---|
| | 25±2°C / 65±5%RH | 40±2°C / 75±5%RH | 25±2°C / 65±5%RH | 40±2°C / 75±5%RH | 25±2°C / 65±5%RH | 40±2°C / 75±5%RH |
| **First day** | 99.48 | 99.48 | 97.6 | 98.6 | 6.6 | 6.6 |
| **30 days** | 99.40 | 99.30 | 99.1 | 97.9 | 6.6 | 6.6 |
| **60days** | 99.31 | 99.2 | 97.2 | 97.1 | 6.6 | 6.6 |
| **90 days** | 98.5 | 98.0 | 98 | 97.8 | 6.6 | 6.6 |

### Relative bioavailability study in pigs

The bioavailability study of optimized buccal tablet (F2) and marketed tablet (immediate release) formulation were conducted in pigs. The mean serum concentration and time profiles of formulations were given in Figure 4. From the results, the $C_{max}$, $T_{max}$ of the F2 and marketed formulations were found to be4.28 ± 0.83 and 2.43 ±0.45 µg/mL; 2.28 ± 0.48 and 0.83 ± 0.25 h, respectively. These $C_{max}$ and $T_{max}$ parameters are twice as compared to thatof marketed formulations and were statistically significant at level of $p<0.01$. There was no statistically significant change in the half-life was observed (3.94 ±0.74 and 3.45 ±0.31 h for F2 and marketed formulation). But, in case of MRT significant changes were observed ($p<0.01$) in F2 (6.98 ±0.37 h) and marketed (4.51±1.24 h) formulations, respectively.

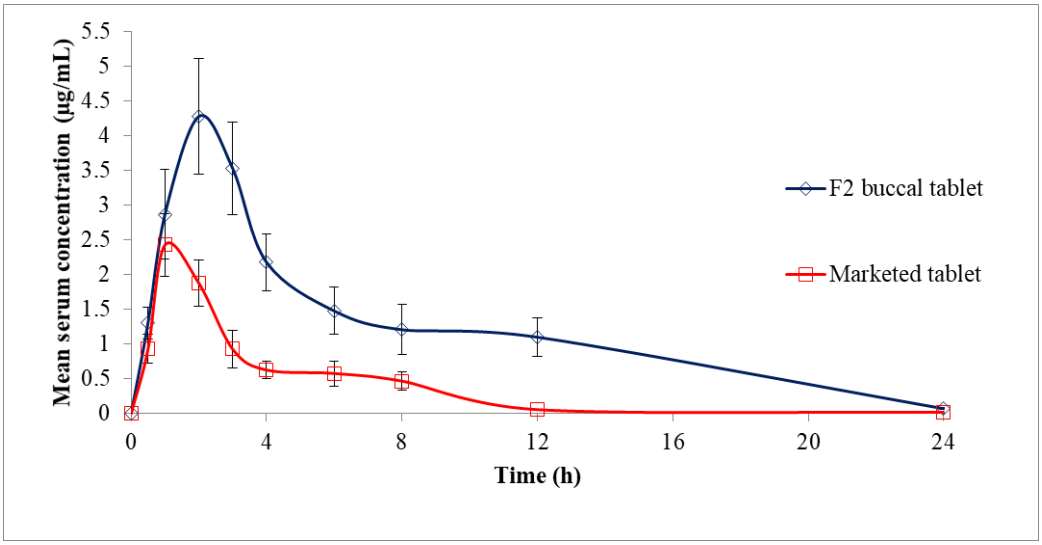


Figure 4. Mean serum concentration-time profiles of F2 formulation and marketed tablets in pigs after oral administration (mean ± SD, n=6)

AUC is the main parameter to depict the bioavailability of the formulation. In this, the $AUC_{total}$ of the F2 buccal and marketed formulation was found to be 27.50 ± 2.17 and 8.89 ± 1.13 µg.h/mL, respectively ($p<0.001$). There was 3.09-folds enhancement in the oral bioavailability of HH from buccal tablet when compared with immediate release marketed formulation. This might be attributed to the peroral administration, where HH is immediately available for absorption; even though, the buccal formulation, analogous to a matrix formulation, has HH surrounded in a polymeric matrix of HPMC $K_4M$. This might cause a slight hindrance in drug release/dissolution from the polymeric matrix prior to being readily available for absorption.

## CONCLUSION

This study was aimed to develop the buccal drug delivery system for HH with controlled effect and to avoid first pass metabolism. From the study, it is observed that formulation F2 was best in terms of drug release, mucoadhesive permeation and *in vivo* performance across the mucosal membrane of the tablet could be described using diffusion controlled mechanism. Hence, it can be concluded that the formulations of HH mucoadhesive buccal tablets are promising one as the controlled drug delivery, improve bioavailability and may be a good candidate for buccal delivery.

## DECLARATION OF INTEREST

It is hereby declared that this paper does not have any conflict of interest.

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