

MUCOADHESIVE BUCCAL TABLETS OF GLIMEPERIDE- FORMULATION AND EVALUATION

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Received: 31 July 2013, Revised and Accepted: 01 Sep 2013

ABSTRACT

Objectives: The present investigation is concerned with formulation and evaluation of Mucoadhesive buccal tablets containing antidiabetic drug, Glimepiride to circumvent the first pass effect and to improve its bioavailability with reduction in dosing frequency and dose related side effects.

Methods: The tablets were prepared by direct compression method. Six formulations were developed with varying concentrations of polymers like Carbopol 934P, HPMCK4M and Chitosan. The tablets were tested for weight variation, hardness, surface pH, drug Content uniformity, percentage swelling index, bioadhesive strength, *ex-vivo* residence time *in-vitro* drug dissolution study, *In-vitro* drug release kinetic study, *ex-vivo* permeation study and Stability study.

Results: FTIR studies showed no evidence on interactions between drug, polymers, and excipients. The best *in-vitro* drug release profile was achieved with the formulation F3 which contains the drug, Carbopol 934p, HPMC K4M and Chitosan in the ratio of 1:3.75:8.75:1.25. The surface pH, bioadhesive strength, *ex-vivo* residence time and swelling index of formulation F3 was found to be 6.80 ± 0.02 , 36.3 ± 0.04 g, 325min and $289.8 \pm 0.52\%$, respectively. The formulation F3, containing 4 mg of Glimepiride exhibited 6 h sustained drug release i.e. $93.98 \pm 0.8\%$ with desired therapeutic concentration. The drug permeation from the formulation F3 was slow and steady and 3.56 mg of Glimepiride could permeate through sheep buccal membrane with a flux of $0.27 \text{ mg hr}^{-1} \text{ cm}^{-2}$. The *in-vitro* release kinetics studies reveal that all formulations fits well with zero order kinetics and followed non-Fickian diffusion mechanism

Conclusion: Hence, it was concluded that the best formulation F3 was suitable for all the evaluation parameters and can be permeated through human buccal mucosa.

Keywords: Mucoadhesive buccal tablets, Bioadhesive strength, *Ex-vivo* residence time, Swelling index, *Ex-vivo* permeation study.

INTRODUCTION

Delivery of drugs via the absorptive mucosa is various easily accessible body cavities, like the ocular, nasal, buccal, rectal and vaginal mucosa has the advantage of bypassing the hepato-gastrointestinal first-pass elimination associated with oral administration[1]. Furthermore, because of the dual biophysical and biochemical nature of these mucosal membranes drugs with hydrophilic and/or lipophilic characteristics can be readily absorbed.

Buccal delivery involves the administration of active pharmaceutical ingredients (API) through buccal mucosa (the lining in the oral cavity)[2,3]. Problems such as first pass metabolism and drug degradation in the gastrointestinal tract acid environment can be circumvented by administering the drug via buccal route[4]. Moreover, the buccal cavity is easily accessible for self medication and drug absorption is terminated in case of toxicity by removing the dosage form from the buccal cavity [5,6]. The API administered through buccal mucosa reaches to the systemic circulation through the internal jugular vein and bypasses the API from the hepatic first pass metabolism, which leads to high bioavailability[7]. Buccal mucosa makes a more appropriate choice of site if prolonged drug delivery is desired because buccal site is less permeable than the sublingual site [8]. In addition, there is excellent acceptability, an expanse of smooth muscle, immobile mucosa, less enzymatic activity, suitable for drugs that mildly and reversibly damage or irritate the mucosa [9]. With the development of mucosal delivery systems having controlled drug release characteristics, the mucosal route can be exploited for the noninvasive systemic delivery of organic and peptide based drugs with rapid absorption as well as sustained drug action[10]. Drug delivery via the buccal route, using bioadhesive dosage form offers such a novel route of drug administration. Buccal delivery involves administration of desired drug through the buccal mucosal membrane lining oral cavity. The buccal route has promising advantages as an alternative to other traditional method of systemic drug administration and hence researchers worldwide have focused interest on development of buccal delivery[11].

The objective of the present research work is to formulate and evaluate mucoadhesive buccal tablets containing Glimepiride as a drug using different ratio's of polymers to avoid hepatic first pass metabolism and to increase bioavailability of drug. Drug like Glimepiride has been selected as model drug because the drug shows promising pharmacokinetics and physicochemical properties required for novel control release dosages. Glimepiride is medium to long acting sulfonyl urea antidiabetic drug. It is classified as first third generation sulfonyl urea. It is used to treat type 2 diabetes mellitus. It acts as insulin secretagogues. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. Glimepiride has Molecular weight of 490.616, protein binding >99.5% and half life of 5hrs [12]. Thus, it was considered as a potential drug for buccal drug delivery.

MATERIALS AND METHODS

Materials

Glimepiride was a gift sample from Dr Reddys Laboratories, Hyderabad. Carbopol 934P was purchased from S.D. Fine chem. Ltd, Mumbai. HPMCK4M was purchased from Yarrow chemicals ltd, Mumbai. Chitosan was a gift sample from Central Drug House, Mumbai. All other reagents used were of analytical grade.

Preformulation Studies

Bulk Density

Bulk density was determined (bulk density apparatus, konark instruments, India) by taking the dried granules in a measuring cylinder and measures the volume and weights of the total granules.

$$\text{Bulk Density} = \frac{\text{Total Weight}}{\text{Total Bulk Volume}}$$

Tapped Density

Tapped density was determined (bulk density apparatus, konark instruments, India) by taking the dried granules in a measuring cylinder and measures the volume of granules after 100 tapping and weight of the total granules.

$$\text{Tapped Density} = \frac{\text{Total Weight}}{\text{Total Tapped Volume}}$$

Compressibility index

Compressibility index was determined by placing the dried granules in a measuring cylinder and the volume (V_0) was noticed before tapping, after 100 tappings again volume (V) was noticed.

$$\text{Compressibility index} = (1 - V_0/V) * 100$$

Where, V_0 = volume of powder/granules before tapping

V = volume of powder/granules after 100 tappings.

Angle of Repose

Angle of repose was determined by measuring the height, radius of the heap of the powder bed. A cut system funnel was fixed to a stand and the bottom of the funnel was fixed at a height of 5 cm from the plane. Powder bed were placed in funnel and allowed to flow freely and measure the height and radius of the heap of powder bed. These studies were carried out before and after incorporating lubricants/ glidants.

$$\tan \phi = h/r$$

Where h = height of heap of granules

R = radius of heap of granules

Hausner's Ratio

Hausner's ratio is a number that is correlated to the flowability of a powder or granular material. Hausner's ratio is calculated by the formula:

$$H = \text{Bulk Density} / \text{Tapped Density}$$

Where, H = hausner's ratio

Drug Excipient compatibility studies

The drug polymer and polymer-polymer interaction was studied by the FTIR spectrometer using Shimadzu 8400-S, Japan. Two percent (w/w) of the sample with respect to a potassium bromide disc was mixed with dry KBr. The mixture was grind into a fine powder using an agate mortar and then compressed into a KBr disc in a hydraulic press at a pressure of 1000psi. Each KBr disc was scanned 16times at 2 mm/sec at a resolution of 4 cm^{-1} using cosine apodization. The characteristic peaks were recorded.

Preparation of Glimepiride Mucoadhesive Buccal Tablets

Buccal tablets were prepared by a direct compression method. Before going to direct compression all the ingredients were screened through sieve no.100. Except lubricant all the ingredients were thoroughly blended in a glass mortar with pestle for 15 min. After sufficient mixing lubricant was added and again mixed for additional 2-3 min. The mixture is compressed using 8 mm flat faced punch on 8 stages rotary tablet compress machine (Rimek Minipress Karnavati Eng. Ltd, Ahmadabad, India). Formulation chart is given in (Table 2). All tablets contained MCC as filler, magnesium stearate as lubricant and mannitol as diluent and bioadhesive polymers with different mixing ratios of carbopol 934P, HPMC K4M and chitosan.

Table 2: List of the ingredients used in the formulae of different Mucoadhesive buccal tablets of Glimepiride

Ingredients(mg)	F1	F2	F3	F4	F5	F6
Glimepiride	4	4	4	4	4	4
Carbopol 934P	5	10	15	5	10	15
HPMC K4M	25	30	35	35	20	5
Chitosan	35	20	5	25	30	35
Mannitol	18	18	18	18	18	18
MCC	55	60	65	55	60	65
Talc	3	3	3	3	3	3
Mg Stearate	5	5	5	5	5	5
Total weight	150	150	150	150	150	150

Evaluation of Glimepiride Mucoadhesive Buccal Tablets

Weight variation

Ten tablets from each formulation (F1 to F6) were weighed using an electronic balance and the average weight was calculated.

Hardness

Tablets require a certain amount of strength or hardness and resistance to friability, to withstand mechanical shocks of handling in manufacture, packaging and shipping. The hardness of the tablets was determined using Monsanto hardness tester. It is expressed in Kg/cm². Three tablets were randomly picked from each formulation and the mean and standard deviation values were calculated.

Friability

Friability is the measure of tablet strength. Roche type friabilator was used for testing the friability using the following procedure. Twenty tablets were weighed accurately and placed in the tumbling apparatus that revolves at 25 rpm dropping the tablets through a distance of six inches with each revolution. After 4 min, the tablets were weighed and the percentage loss was determined.

Thickness

The thickness of three randomly selected tablets from each formulation was determined in mm using a vernier caliper (Pico India). The average values were calculated.

Content uniformity

Ten tablets from each formulation were taken, crushed and mixed. From the mixture 4 mg of Glimepiride equivalent of mixture was extracted thoroughly with 100 mL of pH 6.8 phosphate buffer. The amount of drug present in each extract was determined using UV spectrophotometer at 228 nm. This procedure was repeated thrice and this average was chosen.

Microenvironment (Surface) pH [13]

The microenvironment pH (surface pH) of the buccal tablets was determined in order to investigate the possibility of any side effects *in vivo*. As an acidic or alkaline pH may cause irritation to the buccal mucosa, it was determined to keep the surface pH as close to neutral as possible. The method adopted by Battenberg *et al* was used to determine the surface pH of the tablet. A combined glass electrode was used for this purpose. The tablet was allowed to swell by keeping it in contact with 5 mL of distilled water (pH 6.5 ± 0.05) for 2 h at room temperature. The pH was measured by bringing the electrode in contact with the surface of the tablets and allowing it to equilibrate for 1 min.

Bioadhesion studies [13]

In evaluation of adhesion, it is important to use uniform surfaces that allow the formation of reproducible adhesive bonds. In present study, sheep buccal mucosa was used as a model mucosal surface for bioadhesion testing. Immediately after slaughter, the buccal mucosa was removed from the sheep and transported to laboratory in

tyrode solution and kept it at 40°C. The composition of tyrode solution (g/L) is sodium chloride 8, potassium chloride 0.2, calcium chloride dihydrate 0.134, sodium bicarbonate 1.0, sodium dihydrogen phosphate 0.05 and glucose 1.0.

Fabrication of assembly

The Mucoadhesive forces of the tablets were determined by means of mucoadhesive measuring device shown in Fig. A. The sheep buccal mucosa was cut into strips/pieces and washed with tyrode solution. At time of testing a section of sheep buccal mucosa (c) was secured keeping the mucosal side out, on the upper glass vial (B) using rubber band and aluminium cap. The diameter of each exposed mucosal membrane was 1 cm. The vial with the sheep buccal mucosa (C) was stored at 37°C for 10 min.

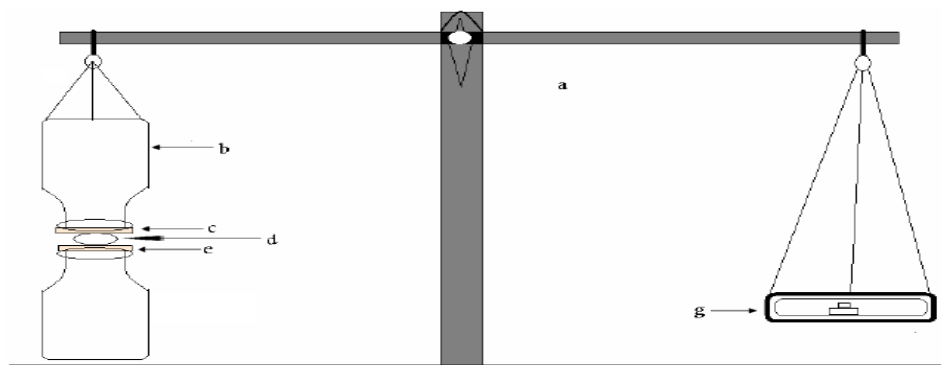


Fig. A: Measurement of bioadhesive strength.

a-scale; b-glass vial; c-sheep buccal mucosa; d-Mucoadhesive tablet; e-adjustable pan; g-weight.

Ex-vivo residence time [14]

The *Ex-vivo* residence time was determined using a modified USP dissolution apparatus. The phosphate buffer of pH 6.8 is used as dissolution medium which is maintained at 37°C ± 2°C. A segment of sheep buccal mucosa each of 4 cm length was glued to the surface of glass slide which was then vertically attached to the apparatus. Three tablets of each formulation were hydrated using 15 µl pH 6.8 buffer on one side and hydrated surface was brought into contact with mucosal membrane. The tablets secured on the glass slide were completely immersed in the buffer solution. The paddle of the dissolution apparatus was 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.

Swelling study [15]

Six Buccal tablets were individually weighed (W1) and placed separately in Petri dishes with 5 mL of phosphate buffer of pH 6.8. At the time interval of 1, 2, 4, 6, 8 and 12 h, tablet was removed from the Petri dish and excess water was removed carefully using the filter paper. The swollen tablet was then reweighed (W2) and the percentage hydration were calculated using the following formula:

$$\text{Percentage hydration} = [(W2-W1)/W1] \times 100$$

In-vitro dissolution studies [16]

The *In-vitro* dissolution study was conducted as per the United States Pharmacopoeia (USP) XXIV. The rotating paddle method was used to study the drug release from the tablets. The dissolution medium consisted of 900 mL of phosphate buffer (pH 6.8). The release was performed at 37°C ± 0.5°C, at a rotation of speed of 50 rpm. 5 mL samples were withdrawn at predetermined time intervals (1 to 6 h) and the volume was replaced with fresh medium. The samples were filtered through Whatman filter paper No.40 and analyzed for Glimperide after appropriate dilution by UV spectrophotometer at 228 nm. The percentage drug release was calculated using the calibration curve of the drug in phosphate buffer pH 6.8

Then one vial with section of sheep buccal mucosa (C) and another vial were fixed on height adjustable pan (E). To a lower vial a tablet (D) was placed with the help of bilayered adhesive tape, adhesive side facing downward. The height of the lower vial was adjusted so that a tablet could adhere to the sheep buccal mucosa on the upper vial. A constant force was applied on the upper vial for 2 min, after which it was removed and the upper vial was then connected to the balance. Then the weight on right side pan was slowly added in an increment of 0.5 g, till the two vials just separated from each other. The total weight (g) required to detach two vials was taken as a measure of Mucoadhesive strength. From this Mucoadhesive strength, the force of adhesive was calculated.

$$\text{Force of adhesion (N)} = \text{Bioadhesive strength}/100 \times 9.81.$$

Drug release kinetic studies [17, 18, 19]

To analyze the mechanism of the drug release rate kinetics of the dosage form, the *in-vitro* dissolution data was fitted into zero-order, first order, Higuchi and Korsmeyer-Peppas release model, to study the drug release from the dosage form.

Evaluation of Optimised Mucoadhesive Buccal Tablet

Ex-vivo permeation studies [14]

Tissue Isolation

Sheep buccal tissue was obtained from a freshly killed sheep (slaughterhouse, Hyderabad) weighing about 12 kg. The tissue was stored in Krebs buffer pH 6.8 at 4 °C and used within 2 hours. The epithelium was separated from the underlying connective tissue with a surgical technique making sure that the basal membrane was still present and the membrane was allowed to equilibrate for one hour in receptor buffer to regain lost elasticity. Slice thickness range from 2.1 to 2.5 mm.

In vitro drug permeation through sheep buccal membrane

In vitro permeation of Glimperide buccal tablet was studied through the sheep buccal membrane. The sheep buccal membrane was mounted between the donor and receptor compartment of the standard Franz diffusion cell with a diffusion area of 2.1 cm² and the acceptor compartment volume of 21ml. The two chambers were tied with the help of springs so that the buccal membrane did not move from its place. The phosphate buffer pH 6.8 in the acceptor compartment was continuously stirred at 600rpm using a magnetic stirrer. The entire setup was placed over a magnetic stirrer and the temperature was maintained at 37 °C by placing the diffusion cell in a water bath. The selected tablet (F3) containing 4mg of Glimperide was placed into the donor compartment. The amount of drug permeated through the membrane was determined by removing aliquots from the receptor compartment and by replacing the same volume of buffer. The amount of Glimperide in the diffusion samples was estimated by the HPLC method.

The flux (J) through the membrane was calculated by using the equation 1.

$$J = dQ / A dt \dots\dots\dots (1)$$

Where J is flux (mg h⁻¹ cm⁻²);

dQ/dt is the slope obtained from the steady-state portion of the curve and

A is the area of diffusion (cm²)

HPLC analysis:

Instrument: youngling instrument

Software: Autochro 3000+

Column: C18, 5µm

Lambda max: 228nm

Temp: 35°C

Injection volume: 20µl

Time -10min

Mobile phase: - Acetonitrile: Phosphate buffer (4:1 ratio)

pH adjusted to 2 with HCl

The results are shown in (Figure 09).

Stability study [20]

The purpose of stability study is to provide evidence on the quality of a drug substance or drug product, which varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. Formulations were selected for

stability on the basis of the *In-vitro* drug release profile. The formulations were subjected to accelerated stability studies as per ICH (The International Conference of Harmonization) guidelines i.e. 25°C/60% RH and 40°C/75% RH in air tight high density ethylene bottles for 2 months in thermo stated ovens. The samples were taken out at 0, 30, 40, 50 and 60 days. Tablets were evaluated for the different physicochemical parameters i.e. content uniformity, surface pH, bioadhesive strength, swelling index and percentage of drug release. The results are shown in (Table 8).

RESULTS AND DISCUSSIONS

Evaluation of pre-compression characteristics of powder bed

The pre-compression characteristics of powder bed was evaluated for all the formulations i.e. F1 to F6. The Angle of repose of powder bed was found to be in the range of 24.40±0.38 to 29.87±0.53 indicates that the powder had excellent flow properties. The Loose Bulk density and Tapped Bulk density were found to be in the range of 0.437±0.011 to 0.488±0.009 and 0.526±0.010 to 0.550±0.008 g/ml respectively which was used to calculated the Carr's index and Hausner's ratio. The values of Carr's index and Hausner's ratio were found to be in the range of 9.50±0.85 % to 19.30±0.22% and 1.105±0.12 to 1.24±0.11 respectively. All the values of flow properties were showed in (Table 1).These trials were conducted in triplicates (n=3).

Evaluation of Glimepiride mucoadhesive buccal tablets

Compatibility studies

The incompatibility between the drug and excipients were studied by FTIR spectroscopy. The spectral data of pure drug and various drug-excipient mixtures are presented in (Figures. 1 to 6). The results indicate that there was no chemical incompatibility between drug and excipients used in formulation.

Table 1: Evaluation of rheological characteristics of powder bed compression for Glimepiride Mucoadhesive Buccal Tablets

Formulation Code	Bulk Density (g/ml)	Tapped Bulk Density (g/ml)	Angle of Repose (°)	Carr's Index (%)	Hausner's Ratio
F1	0.471±0.019	0.531±0.014	24.40±0.38	11.26±0.14	1.127±0.16
F2	0.476±0.012	0.526±0.010	26.56±0.44	9.50±0.85	1.105±0.12
F3	0.488±0.009	0.548±0.013	29.87±0.53	10.87±0.89	1.122±0.18
F4	0.448±0.011	0.528±0.014	25.24±0.39	14.52±0.52	1.17±0.13
F5	0.450±0.013	0.550±0.008	27.06±0.27	18.03±0.37	1.22±0.14
F6	0.437±0.011	0.542±0.012	28.83±0.21	19.30±0.22	1.24±0.11

For all n=3±S.D.

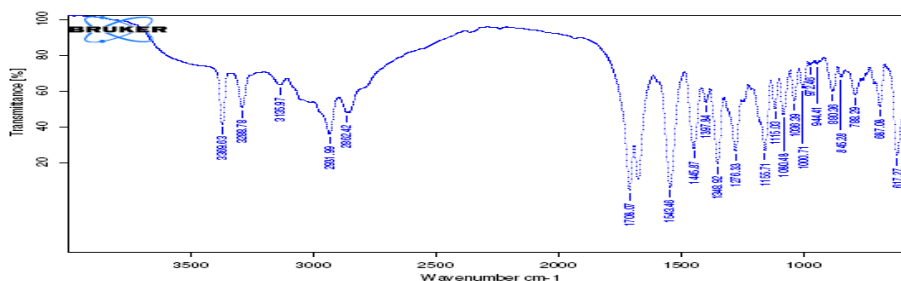


Fig. 1: FTIR Spectroscopy of Glimepiride Pure drug

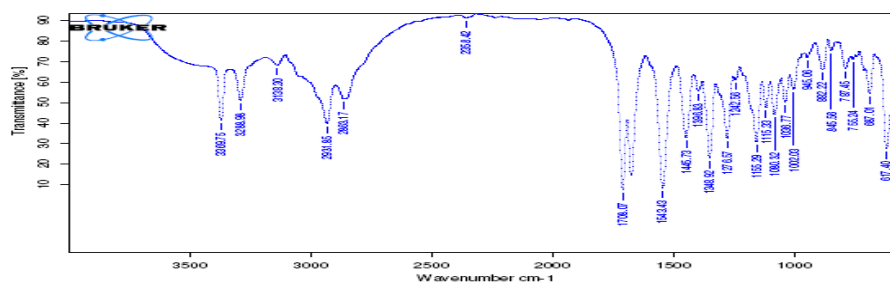


Fig. 2: FTIR Spectroscopy of Glimepiride + Carbopol 934P

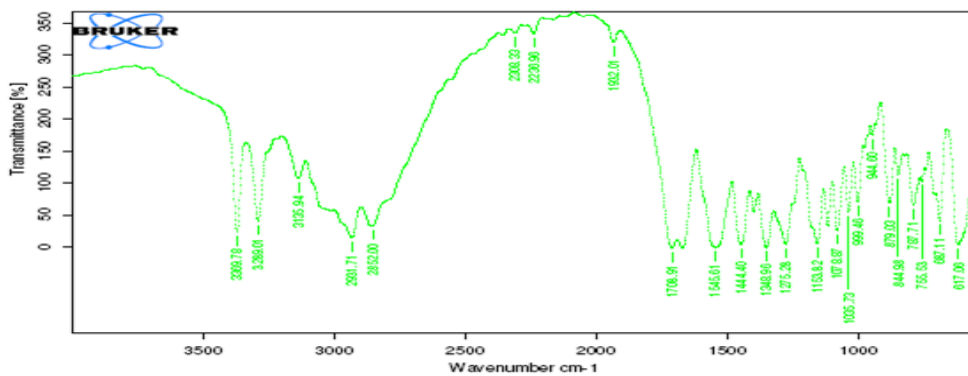


Fig. 3: FTIR Spectroscopy of Glimepiride + HPMCK4M

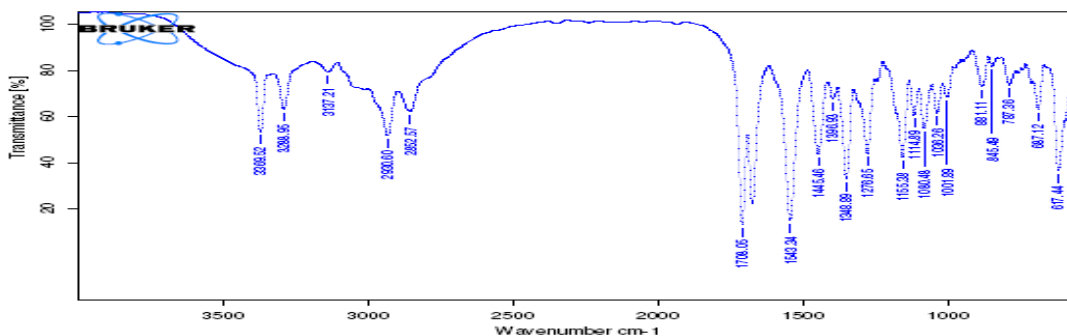


Fig. 4: FTIR Spectroscopy of Glimepiride + Chitosan

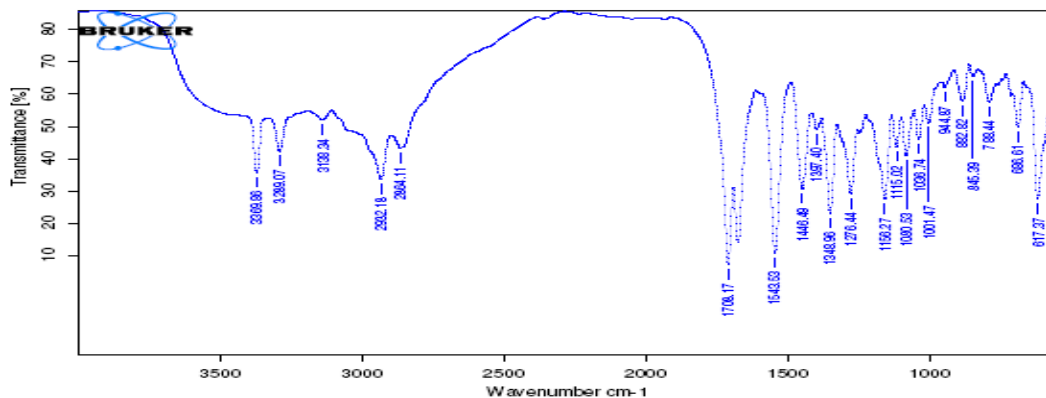


Fig. 5: FTIR Spectroscopy of Glimepiride + Carbopol 934P + HPMCK4M + Chitosan

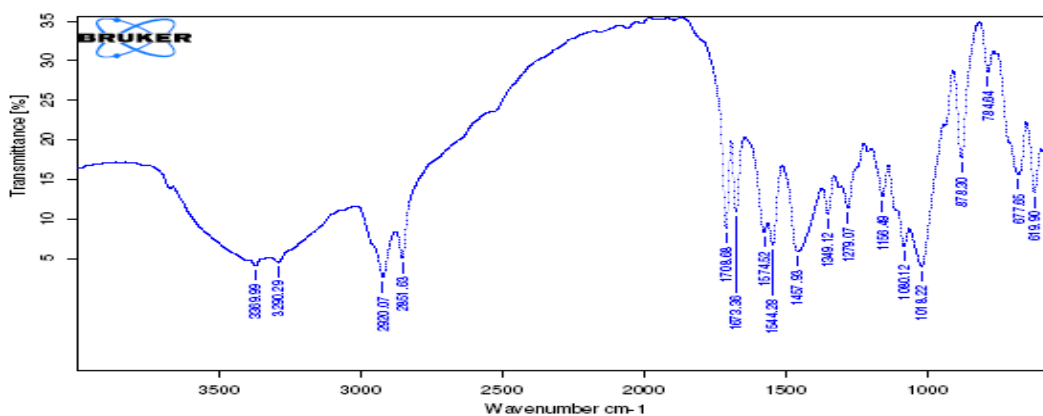


Fig. 6: FTIR Spectroscopy of Glimepiride + Carbopol 934P + HPMCK4M + Chitosan + MCC + Mannitol + Mg Stearate + Talc

Hardness test

The adequate tablet hardness is necessary requisite for consumer acceptance and handling. The measured hardness of the tablets of each batch of all formulations i.e. F1 to F6 were ranged between 4.0 ± 0.09 to 4.6 ± 0.05 Kg/cm² and the results are shown in (Table 3). This ensures good handling.

Thickness

The thickness of the tablets was found to be almost uniform in all formulations F1 to F6. The thickness was found to be in the range of 3.26 ± 0.057 to 3.73 ± 0.010 mm. None of the formulations (F1 to F6) showed a deviation. Hence, it is concluded that all the formulations complied the thickness test and the results are shown in (Table 3).

Weight variation test

The weight variation test was conducted for each batch of all formulations F1 to F6 as per I.P and the results are shown in Table 3. The weight variation test for all the formulations complies with the IP limit ($\pm 10\%$). The weight variations for formulations F1 to F6 were ranged between 149.0 ± 0.20 to 151.1 ± 0.48 mg.

Friability test

The friability test for all the formulations (F1 to F6) were done as per the standard procedure I.P. The results of the friability test were ranged between 0.132 ± 0.05 to $0.533 \pm 0.04\%$ and are tabulated in (Table 3). The data indicates that the friability was less than 1% in all formulations ensuring that the tablets were mechanically stable.

Surface pH

Surface pH of all the formulations F1 to F6 was found to be 6.22 ± 0.08 to 6.87 ± 0.02 , which is well within the limit of acceptable salivary pH range of 5.6 to 7.9. Hence, it was concluded that all formulations could not produce any local irritation to the mucosal surface. The results are shown in (Table 3).

Drug content

The drug content of each batch of all the formulations (F1 to F6) was evaluated as per the standard protocol and the results are shown in the (Table 3). The results indicate that the percentage of drug content was found to be $95.28 \pm 0.18\%$ to $104.12 \pm 0.65\%$. Hence it is concluded that all the formulations are following acceptable limits as per Indian Pharmacopoeia i.e. $\pm 5\%$

Table 3: Hardness, Thickness, Weight variation, % Friability, Surface pH and drug content of Glimepiride Buccal tablets (Formulations F1 to F6)

Formulation code	Hardness (Kg/cm ²)	Thickness (mm)	Wt.Variation (mg)	Friability (%)	Surface pH	Drug content (%)
F1	4.2 ± 0.05	3.73 ± 0.010	149.6 ± 0.25	0.533 ± 0.04	6.67 ± 0.06	104.12 ± 0.65
F2	4.3 ± 0.05	3.68 ± 0.030	150.4 ± 0.60	0.463 ± 0.09	6.43 ± 0.04	101.66 ± 0.75
F3	4.6 ± 0.05	3.68 ± 0.042	150.2 ± 0.50	0.333 ± 0.10	6.80 ± 0.02	102.65 ± 0.71
F4	4.0 ± 0.09	3.26 ± 0.057	151.1 ± 0.48	0.400 ± 0.02	6.87 ± 0.02	97.74 ± 0.69
F5	4.2 ± 0.15	3.40 ± 0.023	149 ± 0.20	0.132 ± 0.05	6.78 ± 0.01	96.75 ± 0.26
F6	4.4 ± 0.10	3.46 ± 0.010	148.7 ± 0.47	0.134 ± 0.02	6.22 ± 0.08	95.25 ± 0.18

For all $n=3 \pm S.D.$

Bioadhesive strength

The *in vitro* bioadhesive strength study was performed by using sheep buccal mucosa and the results are shown in the (Table 4). On the modified physical balance and measure the force (N) required detaching the tablet. The bioadhesion characteristics were affected by the concentration of the bioadhesive polymers. The bioadhesive strength for the formulations F1 to F6 were found to be in the range of 16.16 ± 0.20 gm to 36.3 ± 0.05 gm. The formulations i.e. F1, F2, F3, F4, F5 & F6 with carbopol 934p, HPMCK4M and chitosan showed the bioadhesive strength of 21.0 ± 0.05 gm, 28.6 ± 0.28 gm, 36.13 ± 0.04 gm, 25.3 ± 0.25 gm, 20.3 ± 0.05 gm & 16.16 ± 0.20 gm respectively. From the above

results it was observed that the formulation F6 showed minimum bioadhesive strength i.e. 16.16 ± 0.20 gm, due to increase in the concentration of primary polymer i.e. Carbopol 934P and decreasing and increasing the secondary polymers i.e. HPMCK4M and Chitosan respectively and the formulation F3 showed the maximum bioadhesive strength i.e. 36.3 ± 0.04 gm, due to increase in the concentration of primary polymer i.e. Carbopol 934P and increasing and decrease in the concentration of secondary polymers i.e. HPMCK4M and Chitosan respectively. The Bioadhesive force (N) was conducted for all the formulations i.e. F1 to F6. The maximum bioadhesive force was found to be 3.531 ± 0.06 in formulation F3 and minimum bioadhesive force was found to be 1.569 ± 0.19 in formulation F6.

Table 4: In-vitro Bioadhesive strength and Bioadhesive Force

Formulation Code	Bioadhesive Strength(gm)	Bioadhesive Force (N)
F1	21.0 ± 0.05	2.06 ± 0.06
F2	28.6 ± 0.28	2.746 ± 0.29
F3	36.3 ± 0.04	3.531 ± 0.05
F4	25.3 ± 0.25	2.452 ± 0.21
F5	20.3 ± 0.05	1.962 ± 0.06
F6	16.16 ± 0.20	1.569 ± 0.19

For all $n=3 \pm S.D.$

Ex-vivo residence time

The *Ex-vivo* residence time is one of the important physical parameter of buccal mucoadhesive tablets. The *Ex-vivo* residence time was determined by using specially designed dissolution apparatus. The *Ex-vivo* residence time for the formulations F1 to F6 was found to be in the range of 230 minutes to 325 minutes. The formulations i.e. F1, F2, F3, F4, F5 & F6 with carbopol 934p, HPMCK4M and Chitosan showed the residence time of 260, 290, 325, 230, 240 and 270 min respectively. From the above results it was observed that the formulation F4 showed minimum residence time i.e. 230 min, due to decrease in the concentration of primary polymer i.e. Carbopol 934P and increasing and decreasing the

secondary polymers i.e. HPMCK4M and Chitosan respectively and the formulation F3 showed the maximum residence time i.e. 325 min, due to increase in the concentration of primary polymer i.e. carbopol 934P and increasing and decreasing the secondary polymers i.e. HPMCK4M and Chitosan respectively. This examination reveals the mucoadhesive capacity of polymers used in formulations. The results are shown in (Table 5).

Swelling study

The swelling studies were conducted for all formulations i.e. F1 to F6 and the results were shown in (Table 6). All the formulations were hydrated generally by keeping the tablets in contact with

water for 1 h to 12 h. The highest hydration (swelling) i.e. 289.8±0.41% was observed with the formulation F3. This may be due to quick hydration of polymers (Carbopol 934P, HPMCK4M and Chitosan).

In-vitro dissolution studies

In-vitro dissolution studies were designed to carry out in such a way that they simulate *in-vivo* conditions. The purpose of *in-vitro* release study was to provide a fast, easily performed and in-expensive method that correlates with the performance of dosage form in human subjects. The conditions of *in-vitro* dissolution test were well defined, standardized and enable comparison among various results. For *in-vitro* dissolution study, it was decided to carry out the dissolution in pH 6.8 phosphate buffer. The formulations F1, F2, F3, F4, F5, and F6 containing drug, Carbopol 934p, HPMCK4M and Chitosan polymers in the ratios of 1:1.25:6.25:8.75, 1:2.5:7.5:5, 1:3.75:8.75:1.25, 1:1.25:8.75:6.25, 1:2.5:5:7.5, and 1:3.75:1.25:8.75 respectively. The *in-vitro* cumulative drug release profile of formulations F1, F2, F3, F4, F5, and F6 showed 84.55 ±0.6 %, 89.21 ±0.4 %, 93.98 ±0.8 %, 85.38 ±0.5 %, 90.77 ±0.6 % and 86.69 ±0.8 %, respectively at the end of 6 hours. Among these six formulations, F3

was found to be highest percentage drug release. During the study it was observed that the tablets were initially swell and no erodible over the period of 6 h. It was concluded that by increasing the concentration of carbopol 934p and increasing and decreasing the concentration of HPMCK4M and Chitosan, the drug release rate from the tablets was found to be increased. This may be due to increased hydration (or) swelling of carbopol 934p with increasing concentration.

From the overall data it was found that the formulation F3 showed the maximum percentage of drug release i.e. 93.98±0.8% at the end of 6 hrs. The results are shown in (Figure 07-08).

Table 5: Ex-vivo residence time of formulations F1 to F6

Formulation Code	Ex-vivo residence time (minutes)
F1	260min
F2	290min
F3	325min
F4	230min
F5	240min
F6	270min

Table 6: percentage of hydration of Glimepiride buccal tablets

Time (hr)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	50.6±0.34	48.3±0.09	47.3±0.64	49.01±0.34	46.3±0.87	43.5±0.92
2	96.6±0.31	93.4±0.52	89.9±0.73	82.4±0.55	79.1±0.32	78.3±0.31
4	127.1±0.73	125.3±0.91	123.6±0.29	93.9±0.33	90.6±0.29	89.1±0.22
6	157.6±0.55	154.05±0.49	155.3±0.42	126.6±0.38	124.3±0.33	122.6±0.26
8	193.2±0.92	191.2±0.89	189.4±0.88	156.7±0.47	155.1±0.38	153.3±0.55
12	254.6±0.41	271.7±0.44	289.8±0.52	215.3±0.71	213.5±0.69	211.3±0.62

For all n=3±S.D.

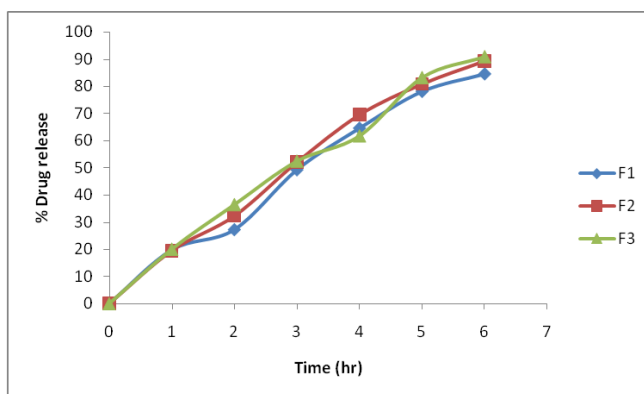


Fig. 07: In-vitro % Drug release profile of formulations F1, F2 & F3

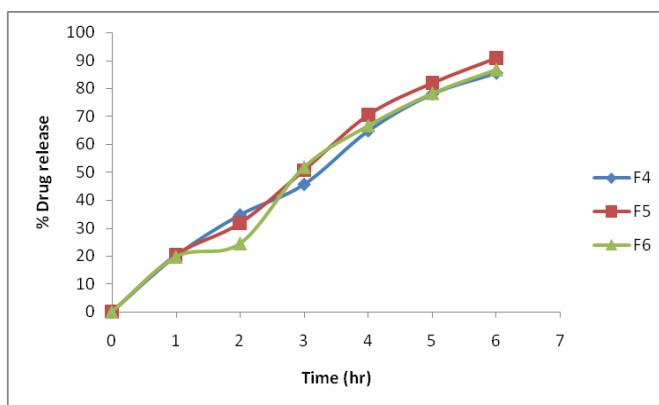


Fig. 08: In-vitro % Drug release profile of formulations F4, F5 & F6

Drug release kinetics

In-vitro drug release data of F1 to F6 were fitted to Zero order, First order, Higuchi and Korsmeyer-Peppas equations to ascertain the pattern of drug release.

In-vitro drug release data for all the formulations F1 to F6 were subjected to release kinetic study according to Zero order, First order, Higuchi and Korsmeyer-Peppas equation to ascertain the mechanism of drug release. Among the Zero-order and First-order,

the R^2 values were found to be higher in zero-order. So all the formulations followed Zero-order kinetics. But in case of mechanism of drug release, between Higuchi and Korsmeyer-Peppas equation, the R^2 value were found to be higher in Korsmeyer-Peppas equation and release exponent "n" value less than 1 i.e. ($n > 0.5$). This indicates that all the formulations followed non-Fickian diffusion. Hence it was concluded that all the formulations followed Zero-order drug release with non-Fickian diffusion. The results are shown in (Table 7).

Table 7: Drug release kinetics of all the Formulations (F1-F6)

Formulation code	Zero order	First order	Korsmeyer-Peppas		Higuchi
	R^2	R^2	R^2	n	R^2
FI	0.984	0.969	0.944	0.784	0.927
FII	0.987	0.963	0.972	0.809	0.939
FIII	0.989	0.927	0.983	0.787	0.942
FIV	0.982	0.977	0.980	0.774	0.952
FV	0.987	0.967	0.972	0.789	0.940
FVI	0.985	0.970	0.960	0.793	0.936

Evaluation of Optimized Mucoadhesive buccal tablets

Ex-vivo Permeation Studies

It was concluded that the formulation F3 containing drug, carbopol 934P, HPMCK4M, Chitosan in the ratio of 1:3.75:8.75:1.25, showed good moisture absorption, Bioadhesive strength, *Ex-vivo* residence time as well as promising drug release pattern. On the basis of above results formulation F3 was studied for *ex-vivo* drug permeation using sheep buccal mucosa. The buccal mucosa of sheep resembles that of humans in terms of structure and composition and therefore

sheep buccal mucosa was selected for drug permeation studies. The optimized formulation was analyzed by HPLC method at 228nm for 6hrs release through sheep buccal mucosa. The flux was calculated.

The results of drug permeation from optimized formulation through the sheep buccal mucosa revealed that Glimepiride was released from the optimized formulation and permeate through the sheep buccal membrane and could possibly permeate through the human buccal membrane. The drug permeation from F3 was slow and steady (Figure 09) and 3.56 mg of Glimepiride could permeate through the buccal membrane with a flux of $0.27 \text{ mg hr}^{-1} \text{ cm}^{-2}$.

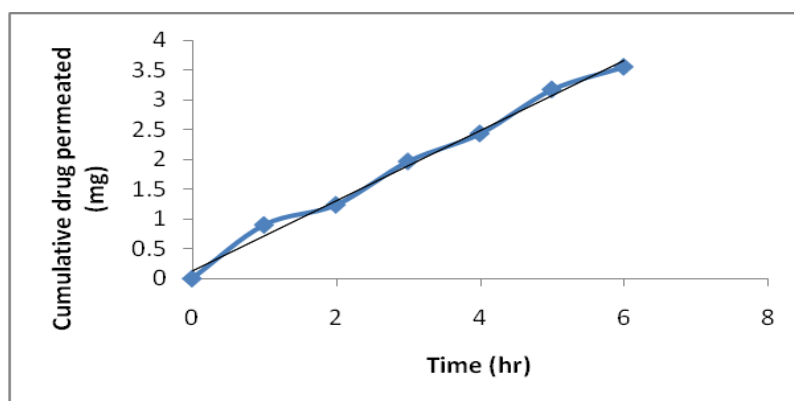


Fig. 9: Ex-vivo permeation of formulation F3

Stability Studies

The stability studies were conducted on the selected formulation F3 as per the ICH guidelines i.e. $25^\circ\text{C}/60\% \text{ RH}$ and $40^\circ\text{C}/75\% \text{ RH}$. The stability studies were done at the intervals of 0, 15, 30 and 60 days. The parameters studied were hardness, percentage drug content,

surface pH, bioadhesive strength, and percentage of drug release. The results are shown in (Table. 8).

From the above results it was concluded that there was no so much significant changes in any values. Hence the formulation F3 was considered to be highly stable.

Table 8: Stability studies of formulation F3 as per ICH guidelines

Characteristics	Initials	15 days	30 days	60 days
	$25^\circ\pm 2^\circ\text{C}$ $60\pm 5\% \text{ RH}$	$25^\circ\pm 2^\circ\text{C}$ $60\pm 5\% \text{ RH}$	$25^\circ\pm 2^\circ\text{C}$ $60\pm 5\% \text{ RH}$	$40^\circ\pm 2^\circ\text{C}$ $75\pm 5\% \text{ RH}$
Hardness (kg/cm ²)	4.6±0.05	4.3±0.05	4.3±0.05	4.3±0.05
Drug Content(mg/tablet)	102.65±0.71	101.12±0.24	100.02±0.73	99.72±0.92
Surface pH	6.80±0.02	6.75±0.03	6.72±0.02	6.70±0.12
Bioadhesive-strength (gm)	38.3±0.04	38.15±0.09	38.05±0.12	37.89±0.06
<i>In vitro</i> drug release	93.98±0.8	91.19±0.3	89.97±0.7	89.02±0.6

For all n=3±S.D.

CONCLUSION

Glimepiride mucoadhesive buccal tablets could be formulated using the drug, Carbopol 934P, HPMCK4M and Chitosan with different ratios. The six formulations i.e. F1 to F6 were evaluated for physicochemical parameters i.e. hardness, thickness, weight variation, friability, surface pH, Bioadhesive strength, *Ex-vivo* residence time, percentage Swelling index, *In-vitro* drug release studies, *In-vitro* drug release kinetic studies, *ex-vivo* permeation studies using sheep buccal mucosa and stability studies. The best formulation F3 was showing the maximum percentage drug release i.e. $93.98 \pm 0.8\%$ at the end of 6 hrs. The *in-vitro* drug release kinetics revealed that all the formulations fits to zero-order kinetics followed by non-fickian diffusion mechanism. The drug permeation from the formulation F3 was slow and steady and 3.56 mg of Glimepiride could permeate through the sheep buccal membrane with a flux of $0.27 \text{ mg hr}^{-1} \text{ cm}^{-2}$. Hence, it was concluded that the best formulation F3 was suitable for all the evaluation parameters and can be permeated through human buccal mucosa.

ACKNOWLEDGEMENT

Authors wish to give thanks to Principal, Malla Reddy College of Pharmacy, Secunderabad, Andhra Pradesh, for providing suitable research laboratory to carry out this project work.

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