Design, development, in vitro evaluation and pharmcokinetic studies of bioadhesive buccal patches for pioglitazone and glimepiride

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ABSTRACT

The buccal film know-how has arisen as an advanced substitute to the other conventional types of drug delivery systems. The buccal mucosa is a suitable route of administration. In this study, a bioadhesive buccal therapeutic system using Glimepiride and Pioglitazone to prolong the residence time at its point of administration. The FTIR studies showed no chemical incompatibility between drugs and excipients. The DSC of the product showed two separate peaks for Pioglitazone and Glimepiride, indicating no potential incompatibility. It was found that the breadth of all buccal films ranges from 0.28 to 0.30 mm. In all formulations, drug content was determined to be in the range of 1.97 to 2.02 mg for glimepiride and 14.95 to 15.01 mg for pioglitazone. Total percentage of cumulative of drug released from all formulations are from 64.12% to 87.0% for glimepiride and 70.0% to 92.2% for pioglitazone. The predicted values by the model agree with actual values. The drugs were released for 12 h, and it was found to be $C_{max}$ for pioglitazone was 467.5 ng/mL and for glimepiride was 65 ng/mL. $T_{max}$ was determined for pioglitazone was three h and for glimepiride was 2.8 h. The drugs are released for 12 h and the AUC of pioglitazone is 4031.25 h ng/mL, and AUC of glimepiride is 505 h ng/mL. Buccal patch for glimepiride and pioglitazone was prepared successfully.

INTRODUCTION

The advantages of the buccal film are low therapy cost, the cosiness of administration and self-medication (Gupta et al., 2009). Paediatric and elderly patients circumvent taking the solid oral preparations such as tablets and capsules due to choking hazard, difficulty in swallowing and do not follow the doctor’s direction (Gupta et al., 2012).

The use of buccal membranes as sites for drug administration has been a topic of interest for long time onwards. The absorption of the drug from the buccal membrane, evading first-pass hepatic metabolism and gastrointestinal drug degradation. This had been attempted by several researchers (Mandeep et al., 2013; Rathbone et al., 1994; Hao and Heng, 2003).

As buccal films are attached to the buccal mucosa, they can be formulated to exhibit local and systemic action. The buccal film may be advanta-
geous over a buccal tablet for flexibility and comfort. The bioavailability will be improved because of circumventing the first-pass metabolism. These dosage forms do not require an expert to administer, economical and has better patient compliance (Kurosaki and Kimura, 2000; Lee et al., 2000).

A buccal film is a dosage form that uses water dissolving polymer that dissolves the drug in mouth and absorbs into blood (Sudhakar et al., 2006).

The main advantage of the buccal film is the large surface area of the buccal membrane that increases absorption of the drug fast when compared to tablets (Joseph et al., 1987). Bioadhesive buccal films had been formulated for local action to cure fungal infections in the oral cavity (Adhikari et al., 2010).

Mechanism of action (MOA)

Glimepiride (GLM) belongs to Sulphonylureas (SU) which increases insulin secretion from beta-cells of the pancreas and recovers both first and second phases of insulin secretion.

MOA

Pioglitazone (PLG) is a thiazolidinedione group that acts as a peroxisome proliferator activating receptor (PPAR)γ agonist that increases whole-body insulin sensitivity. These drugs have beneficial effects on atherogenic diabetic dyslipidemia and recover several atherosclerotic risk markers and lipoproteins. These two drugs act synergistically to treat type 2 diabetes. To develop a formulation based on mucoadhesive patch system (Derosa, 2007).

Objectives

In the present investigation, an attempt is made to fabricate bioadhesive buccal therapeutic system of Glimepiride and Pioglitazone to sustain the residing time at the site of application to enhance the bioavailability.

MATERIALS AND METHODS

Pioglitazone was purchased from Aarathi drug limited, Mumbai, India. Glimepiride was obtained from Hetero Drugs Ltd, Medhak, India. DMSO, HPMC, Man nan, Xanthan gum, Carrageenan were procured from SDSD Fine chemicals (Mumbai, India). Methanol (HPLC grade, Qualigens, Mumbai), MilliQ water was used throughout the analysis.

FT-IR spectra

Separately the FTIR of pioglitazone, glimepiride and buccal film with potassium bromide was done. This mixture was punched to form a pellet. FTIR spectrophotometer (FTIR 8400 S, Shimadzu) is used for this study.

Diffusion Studies

Diffusion studies were conducted in Franz diffusion cells with a volume of 125 mL in the acceptor compartment. These are carried out at 37°C, and 480 rpm for eighth. Samples were withdrawn in fixed timings. First 2 h in pH 1.2 and then pH 6.8 is used. A cellulose membrane with molecular weight cut-off...
### Table 1: CCD

| Independent Variable (Factor) | -Alpha (α) | -1 | 0  | +1 | Alpha (α) |
|------------------------------|------------|----|----|----|-----------|
| Xanthan gum                  | 23.79      | 30 | 45 | 60 | 66.21     |
| Carrageenan                  | 47.57      | 60 | 90 | 120| 132.43    |

### Table 2: Composition of buccal patch

| Sl. No. | Ingredients                  | Per trial |
|---------|------------------------------|-----------|
| 1       | Glimepiride (mg)             | 0.112     |
| 2       | Pioglitazone (mg)            | 0.840     |
| 3       | DMSO (g)                     | 0.224     |
| 4       | Propylene glycol (g)         | 0.560     |
| 5       | HPMC (g)                     | 1.008     |
| 6       | Mannan (g)                   | 0.120     |
| 7       | Xanthan gum (g)              | 0.060     |
| 8       | Carrageenan (g)              | 0.060     |
| 9       | Ethanol 95% (ml)             | 10.000    |
| 10      | Purified Water (ml)          | 10.000    |

### Table 3: CCD of buccal film

| Run   | Xanthan gum | Carrageenan | Design Point |
|-------|-------------|-------------|--------------|
| F-1   | 60.00       | 60.00       | Factorial    |
| F-2   | 66.21       | 90.00       | Axial        |
| F-3   | 30.00       | 120.00      | Factorial    |
| F-4   | 45.00       | 90.00       | Center       |
| F-5   | 45.00       | 132.43      | Axial        |
| F-6   | 23.79       | 90.00       | Axial        |
| F-7   | 45.00       | 90.00       | Center       |
| F-8   | 45.00       | 90.00       | Center       |
| F-9   | 45.00       | 47.57       | Axial        |
| F-10  | 45.00       | 90.00       | Center       |
| F-11  | 30.00       | 60.00       | Factorial    |
| F-12  | 45.00       | 90.00       | Center       |
| F-13  | 60.00       | 120.00      | Factorial    |

Each formulation contains 2 mg of GLIM and 15 mg of P at 12 k Da. was used. The released drug was estimated by HPLC and is given below.

### HPLC method

The method was developed using Shimadzu LCLC which consisted of a column Agilent BDS Hypersil C18 (250 x 4.6mm) with 5 µm particle size, isocratic pump mode.

### Chromatographic conditions

The assay was detected at 225 nm, and the injection volume 10 µL.

A mixture of Buffer: Methanol: Acetonitrile 37:42:21 was used as mobile phase and a flow rate of 1.5 mL/min. A buffer of 0.05M Disodium hydrogen Phosphate was adjusted to pH 3.0. All solutions were degassed by ultra-sonication. The mobile phase was filtered through a 0.45 µ nylon filter before using (Mallu et al., 2011).

### In-vitro bioadhesive strength studies

Bioadhesive quality of the patches was analysed by the marginally adjusted method utilising the porcine gastric mucosa as the model film. The instrument is comprehensively made out of two physical arm balance in which the correct container had been sup-
Table 4: Physical Parameters of buccal film

| Run | Thickness (mm) | Folding Endurance | Swelling Index (2hr) | Bioadhesive strength (g) |
|-----|----------------|-------------------|---------------------|-------------------------|
| F1  | 0.28 ± 0.01    | 95.20 ± 1.72      | 66.20 ± 1.43        | 9.18 ± 0.30             |
| F2  | 0.28 ± 0.02    | 110.20 ± 4.87     | 67.88 ± 1.30        | 9.3 ± 0.41              |
| F3  | 0.28 ± 0.03    | 100.20 ± 1.60     | 65.43 ± 1.52        | 8.23 ± 0.30             |
| F4  | 0.29 ± 0.04    | 98.60 ± 3.83      | 63.02 ± 0.84        | 9.2 ± 0.57              |
| F5  | 0.29 ± 0.05    | 105.00 ± 2.45     | 65.22 ± 0.84        | 9.4 ± 0.27              |
| F6  | 0.28 ± 0.06    | 90.20 ± 2.32      | 60.4 ± 0.83         | 7.5 ± 0.34              |
| F7  | 0.28 ± 0.07    | 95.80 ± 2.71      | 64.88 ± 1.24        | 8.2 ± 0.41              |
| F8  | 0.28 ± 0.08    | 97.40 ± 2.87      | 65.11 ± 0.91        | 8.1 ± 0.36              |
| F9  | 0.28 ± 0.09    | 89.80 ± 2.48      | 54.97 ± 0.90        | 7.0 ± 0.34              |
| F10 | 0.28 ± 0.10    | 98.20 ± 3.32      | 64.45 ± 0.72        | 8.15 ± 0.36             |
| F11 | 0.28 ± 0.11    | 92.00 ± 2.76      | 64.3 ± 0.58         | 7.93 ± 0.31             |
| F12 | 0.28 ± 0.12    | 97.60 ± 3.26      | 63.41 ± 0.48        | 8.18 ± 0.40             |
| F13 | 0.29 ± 0.13    | 112.00 ± 3.41     | 70.01 ± 0.83        | 9.55 ± 0.31             |

Table 5: Release of optimized formula

| Time, hr | % Drug release | Glime | Pio |
|----------|----------------|-------|-----|
| 0        | 0.00           | 0.00  |     |
| 1        | 19.20          |       | 22.30|
| 2        | 21.38          |       | 24.83|
| 3        | 25.25          |       | 30.58|
| 4        | 32.00          |       | 43.46|
| 5        | 40.44          |       | 46.97|
| 6        | 43.00          |       | 55.75|
| 8        | 62.50          |       | 72.00|

Table 6: Verification of experimental values with predicted by software

| Optimized formula | Folding endurance | Swelling index | Bioadhesive strength (g) | Drug (GLIME) % release at 8 hr | Drug (PIO) % release at 8 hr |
|------------------|-------------------|---------------|--------------------------|-------------------------------|----------------------------|
| Predicted        | 117.48            | 70.60         | 9.49                     | 63.57                         | 70.00                      |
| Experimental     | 115.20            | 68.91         | 9.23                     | 63.10                         | 72.23                      |

planted by a detailing holding glass plate and offset a water gathering skillet hanging to one sidearm. The container got a siphon tube from a 10 L bottle that was kept back at a high spot so that water head in the jug, consistently stays over the water gathering dish. The siphon tube bears a stream controlling gadget. Nylon string was utilised to suspend both the glass plate and the dish. An adhesive tissue mounting stage was joined to the focal point of a glass container. Glass measuring utensil was loaded up with phosphate buffer (pH 6.8) to re-enact in-vivo salivation conditions. A stirrer furnished with temperature control was utilised to keep up the temperature of phosphate buffer (pH 6.8) in a glass dish at 37 ± 0.5 °C. The uncovered film surface was dampened with phosphate buffer (pH 6.8) and left for quite a while for hydration and expanding. At that point glass plate (with the film) was kept on the mucosal tissue made sure about on the tissue mounting stage so that films stayed in contact with the mucosa. The entire set up was saved undisturbed for a few moments (preload time) to set up the grip between the film and mucosal tissue. The glass plate (weight 50 g) itself went about as a preload. After the preload time, water gathering dish was suspended to one sidearm and water
Table 7: ANOVA table for variables

| Source               | Sum of squares | DF | Mean Square | F value | Probability |
|----------------------|----------------|----|-------------|---------|-------------|
| Folding Endurance    | 504.43         | 2  | 252.21      | 33.82   | 0.0001      |
|                      |                |    |             |         | (significant) |
| Model (linear)       |                |    |             |         |             |
| A                    | 234.19         | 1  | 234.19      | 31.40   | 0.0002      |
|                      | 270.24         | 1  | 270.24      | 36.24   | 0.0001      |
| Bioadhesive strength |                | 2  | 3.08        | 13.00   | 0.0017      |
|                      |                |    |             |         | (significant) |
| % CDR at 8 h (Pio)   |                | 2  | 22.05       | 7.70    | 0.0095      |
|                      |                |    |             |         | (significant) |
| Model (linear)       |                |    |             |         |             |
| A                    | 4.09           | 1  | 4.09        | 17.28   | 0.002       |
|                      | 2.06           | 1  | 2.06        | 8.71    | 0.0145      |
| % CDR at 8 h (Glime) |                | 2  | 14.91       | 13.35   | 0.0033      |
|                      |                |    |             |         | (significant) |

was included it, by the siphon tube, at a steady pace of 200 drops for every moment until the separation of the film from mucosal surface occurred—the water gathering dish to hold it at the hour of separation. Weight of water gathered in the container at the hour of separation was estimated. The examination was acted (n = 3). Bioadhesive quality of buccal movies was estimated on the 'Adjusted Physical Equalisation'. Rodent peritoneal film as a model mucosal layer (Sharma et al., 2019).

**In vivo studies**

Healthy rabbits weighing 1.5 to 2.0 kg each were used for the pharmacokinetic studies Institutional Animal Ethics Committee’s approval (ref no. VIPS/1454/12-13) was obtained before the commencement of the study. New Zealand Rabbits (6) were fasted 12 h before the study. A blank blood sample was taken from the marginal ear vein of each rabbit (control). Animals were anaesthetised by giving ketamine (40 mg/kg) and xylazine (5 mg/kg) through the intramuscular route. Then the buccal film was wetted with a small quantity of water (30 mL) and attached to the buccal mucosa of the rabbits. Blood samples were collected at the intervals of time (0, 1, 2, 3, 4, 6, 8, 10, and 12 h).

**Analysis of plasma samples**

The HPLC system is Shimadzu. The column kept at ambient temperature. The mobile consisted of a mixture of methanol and ammonium acetate buffer (pH 3.5) fixed in the ratio of 55: 45 at a flow rate of 0.5 mL/min.

**Extraction of drugs from rabbit plasma**

The extraction of drugs from was done by using ether as a solvent.

The HPLC system consisted of injector valve with a 10 µL loop, the column used was Aqua RP-C18 column (250 x 4.6 mm internal diameter, 5 µm particle diameter), (Phenomenex, USA) and a pre-column (guard column with C18 pre-column inserts) (Waters, USA), a Knauer Model K-2500 UV variable wavelength detector; the chromatograms were saved, the eluent was filtered through a 0.45 µm membrane filter using vacuum filtration unit (Phenomenex, USA). 20 µL aliquots were injected (n = 3) and eluted with the mobile consisted of a mix-
ture of methanol and ammonium acetate buffer (pH-3.5) fixed in the ratio of 55: 45 at a flow rate of 0.5 mL/min the eluent was monitored at 252 nm (Lakshmi et al., 2009).

Figure 1: FTIR spectra of blue -pioglitazone, red -glimepiride and green- buccal film

Figure 2: DSC thermogram of A) glimepiride, B)pioglitazone HCl, and C) buccal film (optimized formula)

Figure 3: Chromatogram for release of drugs (sample)

RESULTS AND DISCUSSION

FTIR

The characteristic bands: Pioglitazone: 3364, 3084, 2928, 1743, 1616, 1460, 1242, 1084, 850 Glimepiride: 1345, 1153, 1708, 1674. Both individual pure drugs and final product spectra were matched (Figure 1).

Figure 4: Actual and predicted values for folding endurance

Figure 5: 3D graph of folding endurance with factors carrageenan and xanthum gum

Figure 6: Actual and predicted values for bioadhesive strength

Figure 7: 3D graph of bioadhesive strength with factors carrageenan and xanthum gum
Inference
No chemical incompatibility between drugs and excipients.

Differential scanning calorimetry (DSC)
Inference
DSC of the product has two separate melting peaks for Pioglitazone and Glimepiride – No potential incompatibility (Figure 2).

Evaluation of buccal patch
Film thickness
It was found that the thickness of all buccal films ranges from 0.28 to 0.29 mm in all formulations. The physical parameters of buccal films were found and were given (Table 4).

Content
The assay for drug content was to determined, and those are in the range 1.97 to 2.02 mg for GLIM and 14.95 to 15.01 mg for PLG. The example of the chromatogram is given in Figure 3.

Effect of variables on folding endurance
The linear model F-value of 33.82 and probability value 0.0001 implies the linear model is affecting significantly. The coded equation gives the constant and regression coefficient for folding endurance, \( R_1 = 98.48 + 5.41\times\text{Xantham gum} + 5.81\times\text{Carrageenan} \). The predicted vs actual and are given in Figures 4 and 5.

**Bioadhesive Strength**

Effect of variables on bioadhesive strength, the constant and regression coefficient for bioadhesive strength is given by \( R_3 = 8.51 + 0.7153\times\text{Xantham gum} + 0.508\times\text{Carrageenan} \). The linear model F-value of 13.00 and probability value 0.0017, which is less than 0.05 means the model is significant. 3D graph and predicted vs actual are given for bioadhesive strength (Figures 6 and 7).

**Effect of variables on release**

The linear model is found significant for drug release with model F-value 13.35 and p-value 0.0015 for glimepiride. Model equation \( R_4 \) (glimepiride 8 h release) = 64.29 - 0.8246\times\text{Xanthan gum} – 1.75\times\text{Carrageenan} . The linear model is found significant for drug release with model F-value 7.70 and p-value 0.0095 for pioglitazone. Model equation \( R_5 \) (pioglitazone 8 h release) = 74.10 - 0.5195\times\text{Xanthan gum} – 2.29\times\text{carrageenan} . The 3D graphs and actual vs predicted graphs for 8\(^\text{th}\) h release of both drugs are given (Figures 8, 9, 10 and 11).

**Optimisation**

1. The composition of the optimised formula is GLIME (2 mg), PIO (15 mg), Xanthan gum (57.37 mg), Carrageenan (120 mg).
2. The percentage of cumulative drug release of the optimised formula is given in Table 4. A good relationship is observed between experimental and predicted values as below (Table 5).

**ANOVA, Pure Error, Absence of Fit**

Results of ANOVA (Table 7) demonstrate model is significant for all dependent variables. All the independent variables (factors) found to be significant for all R1, R2, R3 and R4 response variables. The absence of fit F-value of 5.97 implies (folding endurance) there is a 5.27% coincidental that an absence of Fit F-value this large could occur due to noise. The absence of Fit F-value of 1.24 implies (for bioadhesive strength) not substantial relative to the pure error. Non-significant lack of fit is good – we want the model to fit.

**In vivo evaluation**

The drugs were released for 12 h and it was found to be \( C_{max} \) for pioglitazone is 467.5 ng/mL and for glimepiride is 65 ng/mL. \( t_{max} \) for pioglitazone was found to be 3 h and for glimepiride was 2.8 h (Figure 12). AUC of pioglitazone is 4031.25 h ng/mL and AUC of glimepiride is 505 h ng/mL.

**CONCLUSIONS**

Effect of Xanthan Gum is more pronounced than that of Carrageenan. The experimental independent variables found to be very close to predicted values of the optimised formulation, which proves the possibility of the optimisation method in the successful development of buccal films containing Glimepiride and Pioglitazone.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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