FORMULATION AND EVALUATION OF MUCOADHESIVE BUCCAL PATCHES OF TIMOLOL MALEATE

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ABSTRACT: ***Timolol maleate is a non-selective beta-adrenergic blocker and has a short biological half-life, approximately 4.1 h, and low oral bioavailability. Therefore, the present investigation is concerned with the development of the buccal mucoadhesive patches, which were designed to prolong the residence time and thus improve the bioavailability of the drug and its half-life. Hydroxypropyl methylcellulose (HPMC) is one of the polymers which is having good mucoadhesive properties so, therefore, various formulations were developed by using release rate controlling and gel-forming polymers like HPMC K and Ethylcellulose by solvent casting method. In addition to this glycerol and DMSO was used as plasticizer and permeation enhancer respectively. All the formulations had a good physical appearance and physicochemical properties. From among all the developed formations, since formulation F2 retarded the drug release in a controlled manner for a prolonged period of more than 6 h, gave satisfactory bio adhesion a maximum duration of 6 h, and the drug diffused up to 80%, so it was selected as the best formulation. Swelling studies indicated significant water uptake and contributed to drug release. The most satisfactory formulation had shown no significant change in physicochemical properties, drug content, bio adhesion properties, in vitro diffusion pattern after storage at 30 ± 2ºC (65% RH) and 40 ± 2ºC (75% RH) during stability studies for 3months as per ICH guidelines. Thus, the best formulation satisfied physicochemical parameters, in vitro bioadhesion strength, in vitro drug release, and requirements for a buccal mucoadhesive drug delivery system.***

Keywords: Buccal delivery system, Timolol Maleate, polymers, solvent casting technique, Mucoadhesive patches, In-vitro release studies.

I. INTRODUCTION

1.1. Buccal Drug Delivery System

The unique environment of the oral cavity offers its potential as a site for drug delivery. Because of the rich blood supply and direct access to the systemic circulation, the oral mucosal route is suitable for drugs, which are susceptible to acid hydrolysis in the stomach or which are extensively metabolized in the liver (first-pass effect).[1]

The total area of the oral cavity is about 100 cm^2. Out of this, about one-third is the buccal surface, which is lined with the epithelium of about 0.5 mm thickness. The oral mucosal surface is constantly washed by the saliva[16] (daily turn out is about 0.5 to 2 liters). The continuous secretion of saliva results in rapid removal of the released drugs. Conversely, the thin mucin film, which exists on the surface of the oral mucosa, may provide an opportunity to retain a drug delivery system in contact with the mucosa for prolonged periods if it is designed to be mucoadhesive. [2] Such systems ensure close contact with the absorbing membrane, thus optimizing the drug concentration gradient across the biological membrane and reducing the differential pathway. Therefore, the buccal (oral) mucosa may be a potential site for controlled or sustained drug delivery.[3]

II. MATERIALS AND EQUIPMENT

Table-: List of Materials used

S.NO	MATERIALS	SUPPLIER
1	*Timolol maleate*	*Hetero labs, HYD*
2	*HPMC K 100M*	*AR chemicals*
3	*Ethylcellulose*	*AR chemicals*

4	*Methanol (ml)*	*AR chemicals*
5	*Polyethylene glycol*	*AR chemicals*
6	*DMSO*	*AR chemicals*

Table-: List of equipment used

S. NO	***EQUIPMENT NAME***	***SOURCE***
1	*Digital weighing machine*	*Shimadzu aty 244*
2	*UV-Visible double beam spectrophotometer*	*Lab India UV visible double beam spectrophotometer*
3	*Franz diffusion cell*	*AR chemicals, HYD*
4	*Magnetic stirrer*	*Erweka*
5	*Bath sonicator*	*Wensar*

III. METHODOLOGY

Preformulation Studies

Preformulation involves the application of biopharmaceutical principles and physicochemical parameters of drug substances were characterized to design an optimum drug delivery system. It is an important part of the drug development process. The information relating to drug development acquired during this phase is used for making critical decisions in further stages of development. Characterization of the drug is a very important step at the preformulation phase of product development followed by studying the properties of excipients and their compatibility.

Methods of API characterization

Physical properties

Color

A small amount of Timolol maleate powder was taken on butter paper and viewed in an illuminated place. It appears as a white crystalline powder.

Taste and odor

A very less quantity of Timolol maleate was used to get a taste with the help of tongue as well as smelled to get an odor. The taste was found to be bitter and a characteristic odor.

Solubility studies

Solubility study of Timolol maleate was performed in Water, methanol, ethanol, and pH 6.8 phosphate buffer.

Determination of melting point:

M. P of Timolol maleate was estimated by the capillary method.

Preparation of phosphate buffer pH 6.8:

28.85 gms of disodium hydrogen orthophosphate and 11.45gm of potassium dihydrogen phosphate were weighed to it sufficient water was added to get 1000 ml and the pH was altered to 6.8 with Orthophosphoric acid.

Standard curves of Timolol maleate :

Standard graph of Timolol maleate in phosphate buffer 6.8:

A standard stock solution of Timolol maleate (1mg/ml) was prepared by dissolving 100mg of Timolol maleate in 100ml of methanol. Diluting the standard stock solution with phosphate buffer 6.8, a solution of 100 µg/ml concentration was prepared. From this solution, serial dilutions were made with phosphate buffer 6.8 to get 10, 20,

30, 40, 50 µg/ml concentrations. These solutions were checked for absorbance using UV- Visible spectrophotometer at λ_{max} against phosphate buffer 6.8 as the blank and standard graph was plotted by taking concentration on X-axis and absorbance on Y- axis.

Drug- excipient compatibility study

The compatibility of drug and formulation components is an important prerequisite for formulation development. It is therefore necessary to confirm that the drug does not interact with excipients under experimental conditions and affects the shelf life of the product or any other unwanted effects on the formulation.

The goal of excipient compatibility studies are:

- To identify excipients that are compatible with the active ingredient which does not have any impact on the stability of the active ingredient.
- To assign a relative risk level to each excipient within a functional class.
- To expect a stabilizer to interpose at these points of contact on a random basis is rather simplistic. Because solid-state reactions are generally heterogeneous reactions that occur only at points of contact between drug and excipients.

Method

Compatibility studies were conducted to investigate and predict physicochemical interaction between drug substance and excipients and therefore to select suitability of chemically compatible excipients. Compatibility studies were performed by preparing compatibility blends at different ratios of different excipients with drug based on tentative average weight. These blends were stored at accelerated conditions at 40^0c, 75%RH for one month. The control samples were stored at 40c the ratio of drug and excipient varies from 11 to 110 depending on the purpose of use and samples were kept in double lined poly bags .the samples were evaluated for any change in physical characteristics. Samples were evaluated for any change in physical characteristics with reference to controlled sample stored at 4oc for 30 days. Taken out at two weeks interval and were subjected to physical and chemical testing and results were noted. Chemical compatibility is tested by FTIR spectrometry, which is most powerful technique to identify functional groups of the drug.

Formulation Development

Table-: Formulation Design of Timolol maleate buccal Patches

S. No	F.Code	Ingredients (mg)				
		Drug (mg)	Ethyl cellulose	HPMCK100M	PEG	DMSO
1	F1	20	40	-	1ml	0.1ml
2	F2	20	80	-	1ml	0.1ml
3	F3	20	-	40	1ml	0.1ml
4	F4	20	-	80	1ml	0.1ml

Characterization of Buccal formulation

Physico- chemical evaluation

Physical appearance:

All the formulated Timolol maleate films were observed for color, clarity, flexibility, and smoothness.

Folding endurance:

Buccal patches folding endurance was estimated by frequently double over at the same place till it broke. The number of times the film could be folded at the same place without breaking is the folding endurance. This was restate on all the films for three times and the mean values plus standard deviation was calculated.

Thickness of the film:

The thickness of each film was measured by using screw gauze. Buccal patches thickness was estimated at various sites on each patch and the average thickness of the Buccal patch was capture as the thickness of the patch.

Weight uniformity:

The formulated Buccal patches are to be dried at 60^0C for 6 hours before trial. A identify the area of 4.52 cm^2 of film is to be cut in different parts of the patch and weigh in digital balance. The average weight and standard deviation values are to be calculated from the individual weights.

Drug content :

The formulated Buccal patch were assayed for drug content in each case. patches from each formulation were assayed for content of drug. Each formulation was casted in triplicate and one patch from each was taken and assayed for content of drug.

The Buccal films (4.52 cm^2) were added to conical flask containing 100 ml of phosphate buffer pH 7.4 contain 0.5% SLS. This was then stirred with magnetic bead at 400 rpm for 2 hrs. The contents were filtered and the filtrate was analyzed spectrophotometrically. Similarly a blank was prepared from Buccal films without drug.

Moisture absorption studies:

The buccal patches were weighed exactly and placed in a desiccators containing aluminium chloride to maintain 79.50% RH. After 3 days, the films were taken out and weighed. The percentage of moisture uptake was calculated using the following formula.

$$\text{Perentage moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Moisture loss studies:

Three patches were weighed separately and kept in a desiccator contains calcium chloride at 37^0C for 24 hours. Then the last weight was noted when there was no further change in the weight of the patch. The percentage of moisture loss was calculated using the following formula.

$$\text{Percentage moisture loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Final weight}} \times 100$$

Swelling study.

Three buccal patch were weighed individually (W1) and placed separately in 3% agar gel plates and incubated at 37 ± 1°C. After every 15min time interval until 1 h, the patches were removed from the Petri dish and excess surface water was removed carefully with blotting paper. The swollen patch was then reweighed (W2) and the swelling index (SI) were calculated using the formula given in equation.

[Swelling Index = [(W2-W1) ÷ W1] × 100,

Where W1 = initial weight of the patchW2 = final weight of the patch

Stability studies:

Optimized medicated buccal films were subjected to short term stability testing. The Buccal films were sealed in aluminium foils and kept in a humidity chamber maintained at 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months as per ICH guidelines.

IV. RESULTS AND DISCUSSION

Active pharmaceutical ingredient characterization

Physical properties

The colour odour, taste of the drug was recorded using descriptive terminology.

Properties	results
Description	Crystalline Solid
Taste	tasteless
Odour	odourless
Colour	white

Solubility studies

Solubility study of Timolol maleate is Slightly soluble in water; freely soluble in ethanol

Melting point determination:

The results of partition coefficient and melting point are tabulated in table.

Table-: Determination of Melting point

Melting point (^{o}C)
202 ^{o} C

Determination of λ_{max} using UV- Visible spectrophotometer:

Timolol maleate exhibits absorption maxima at 294 nm in phosphate buffer 6.8

Determination of absorption maxima (λ_{max}) for Timolol maleate

A 10mcg/ml standard solution of Timolol maleate was scanned on a double beam spectrophotometer against respective media blanks. An absorption maximum (λ_{max}) of 294 nm was obtained for all solutions and was selected to prepare standard curve.

Table-: Calibration curve of Timolol maleate

S. no	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.132
3	20	0.234
4	30	0.327
5	40	0.435
6	50	0.543

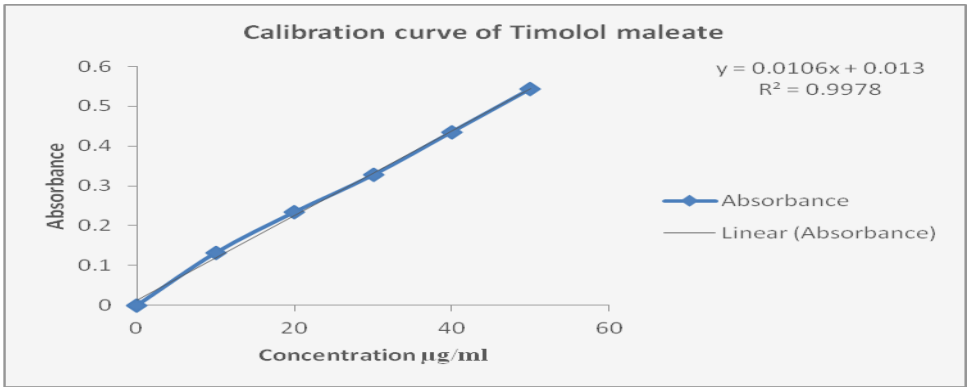

Fig.1. Calibration curve of Timolol maleate

Compatibility studies of drug and polymers:

All these peaks have appeared in formulation and physical mixture, indicating no chemical interaction between Timolol maleate and polymer. It also confirmed that the stability of drug during microencapsulation process.

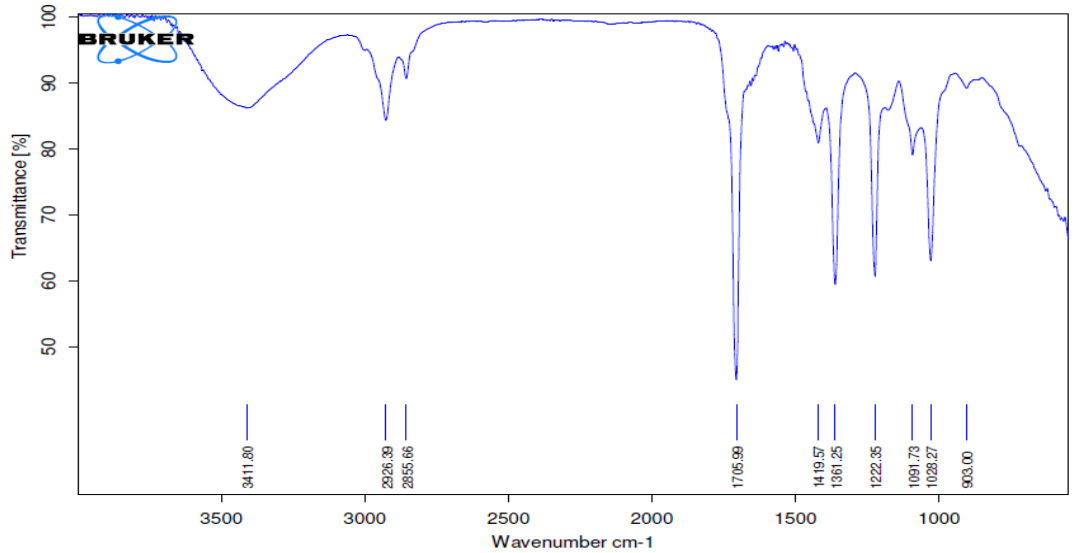

Fig.2. FTIR Studies of Timolol maleate

Table-: Characteristic Peaks for Timolol maleate

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3500-3000	3411.80
2	OH Bending	1000-1500	1222.35
3	C-H stretching	3000-2500	2926.39
4	C=O stretching	2000-1500	1706.99

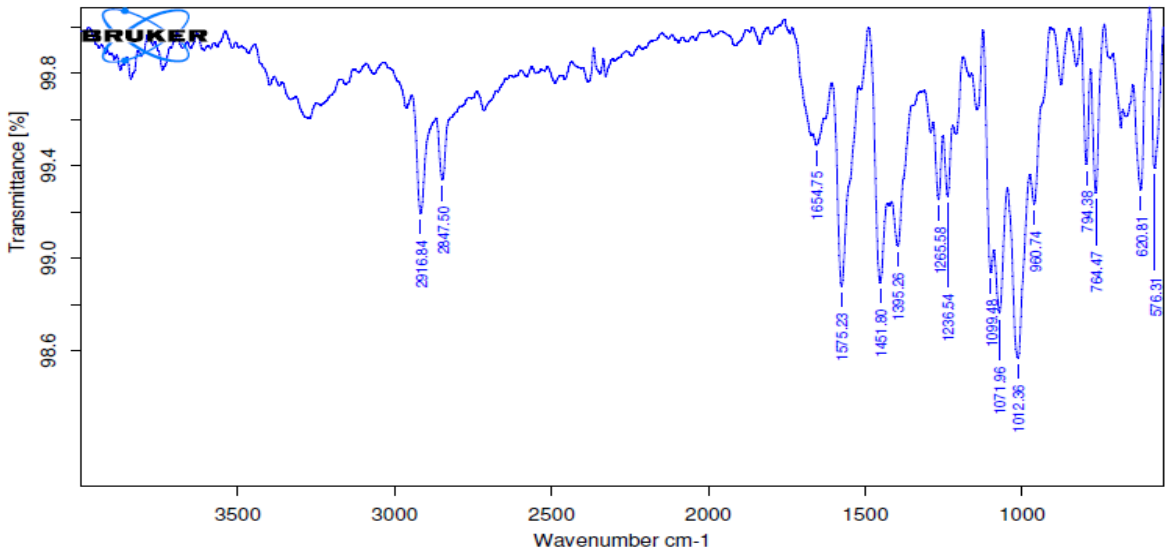

Fig.3. FTIR Studies of Physical mixture of drug and excipients

Table-: Characteristic Peaks for Physical mixture of drug and excipients

S.No.	Characteristic Peaks	Frequency range (cm-1)	Frequency (cm-1)
1	OH stretching	3000-2500	2916.84
2	OH Bending	1100-1070	1071.96
3	C=O stretching	2000-1500	1575.23

Physical appearance and surface texture of buccal patches:

These parameters were checked simply with visual inspection of patches and by feel or touch. The observation reveals that the patches are having smooth surface and they are elegant in appearance.

Weight uniformity of buccal patches:

The weight of the patches was determined using digital balance and the average weight of all patches

Thickness of buccal patches:

The thickness of the patches was measured using screw gauge and the average thickness of all patches.

Folding endurance of buccal patches:

The folding endurance gives the idea of flexible nature of patches. The folding endurance was measured manually, patches were folded repeatedly till it broke, and it was considered as the end point. The folding endurance was found optimum and the patches exhibited good physical and mechanical properties and the average folding endurance of all patches.

Drug content uniformity of buccal patches:

Timolol maleate buccal patches prepared with various polymers were subjected to the valuation for uniform dispersion of drug throughout the patch. In each case three patches were used and the average drug content was calculated.

% moisture loss:

The moisture content in the buccal patches ranged from 8.75 to 8.96%. The moisture content in the formulations was found to be increased by increase in the concentration of polymers.

%moisture absorption:

The moisture absorption in the buccal patches ranged from 9.92 to 10.52%.

Swelling index:

The swelling index in the buccal patches ranged from 14.58 to 15.98 %.

Table -: Physicochemical evaluation data of Timolol maleate Buccal Patches

F. code	F1	F2	F3	F4
Thickness (mm)	0.34	0.24	0.26	0.22
Weight variation (mg)	47.36	48.16	44.62	43.29
Drug content Uniformity	90.96	94.36	94.46	98.83

Folding endurance	76	79	82	88
% moisture loss	8.16	8.82	8.92	8.57
%moisture absorption	10.62	10.74	10.72	10.32
Swelling index	14.89	15.54	15.86	15.45

Drug release studies

Table-: *In vitro* release data of film F_1 to F_4

Time (hrs.)	F_1	F_2	F_3	F_4
0	0	0	0	0
1	26.50	25.55	26.70	25.89
2	44.50	47.59	48.18	45.23
3	67.65	66.55	69.75	68.35
4	71.98	75.32	76.89	70.34
5	85.32	80.28	88.86	86.77
6	90.12	91.22	92.45	95.50

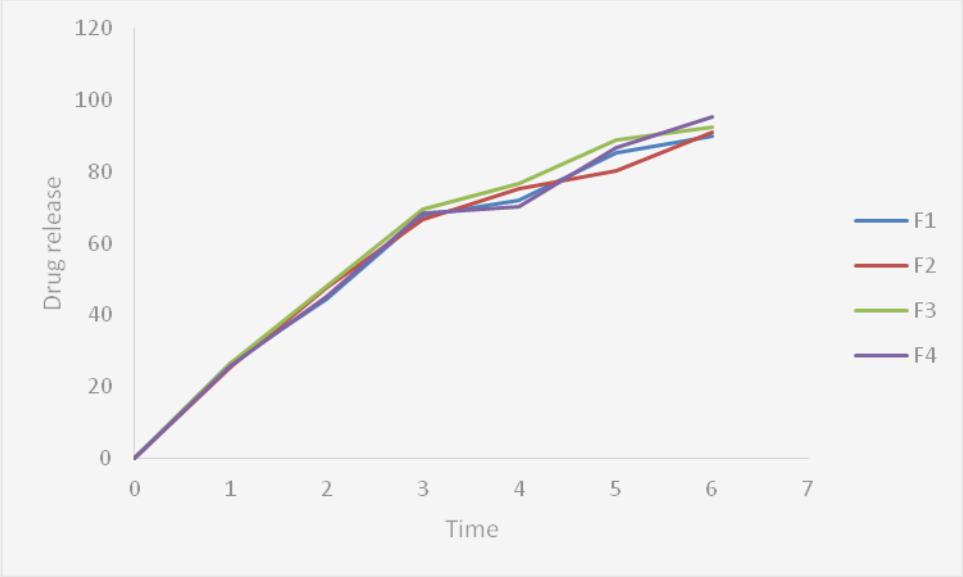

Fig.4. In vitro drug release of (F1- F4) formulation

Stability studies:

Optimized formulations F4 was selected for accelerated stability studies as per ICH guidelines. The patches were observed for color, appearance and flexibility for a period of three months. The folding endurance, weight, drug content, % cumulative drug release of the formulation was found to be decreasing. This decrease may

be attributed to the harsh environment (40^0C) maintained during the studies.

Table-: Stability studies of optimized formulations

S.NO	Time in days	Physical changes	Mean % drug release		
			Timolol maleate		
			25^0C/60%	30^0C/75%	40^0C/75%
1.	01	No Change	95.50	95.50	95.50
2.	30	No Change	94.15	94.09	93.99
3.	60	No Change	94.10	93.95	93.52
4.	90	No Change	93.99	93.52	93.15

V. SUMMARY & CONCLUSION

From this study it was concluded that the buccal patches containing Timolol maleate can be successfully prepared by using release rate controlling polymers. Hence these formulations of Timolol maleate buccal patches with having good permeability.

In the present study it can be concluded that,

• FTIR studies revealed that there is no incompatibility or interaction between Timolol maleate and excipients.

• Formulated buccal films gives satisfactory film characteristics like physical appearance, surface texture, weight uniformity, thickness uniformity, folding endurance, surface pH, percentage swelling index, percentage moisture uptake, drug content uniformity, in-vitro drug release. The low values for standard deviation for average weight ,thickness, surface pH, percentage swelling index, percentage moisture uptake, in vitro drug release and drug content indicated uniformity within the batches.

• Based on in vitro drug release, formulation F2 exhibited a drug release of 95.50% in 6 hours. The drug release could be retarded more than 6 hr with controlled release behavior. The prepared buccal patches were found to stable after performing stability testing for three month.

• Short term stability studies of optimized formulation as per ICH guidelines indicated that there is no significant change in physical appearance, drug content determination and in vitro drug release.

So finally, it can be concluded that mucoadhesive buccal films of Timolol maleate could provide sustained buccal delivery for prolonged period. A further clinical investigation has to be conducted to establish the safety and efficacy of the developed formulation.

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