IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
February 2021 Vol.:20, Issue:3

Development and Evaluation of Buccal Patches of Clopidogrel Bisulphate for the Treatment of Thrombosis

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

S.P. Senthil[1], A. Priyanga*, M. Sakthivel[1], R. Senthamarai[2]

[1]Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamilnadu, India – 620021 [2]Department of Pharmacognosy, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamilnadu, India - 620021

Submitted: 10 January 2021
Revised: 30 January 2021
Accepted: 19 February 2021

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Antiplatelet, Clopidogrel Bisulphate, Buccal patch, Solvent casting method

ABSTRACT

Antiplatelet therapy is the cornerstone in the modern therapy of patients with acute coronary syndrome (ACS), because of the unique role of platelets in coronary thrombosis. Clopidogrel is a thienopyridine which inhibits ADP-induced platelet aggregation, with no direct effects on the metabolism of arachidonic acid. The drug plays a key role in patients undergoing stenting. In order to prevent stent thrombosis. Pretreatment and long term treatment with clopidogrel reduces by about one-third the risk of cardiovascular death or myocardial infarction in NESTEMI-ACS patients undergoing percutaneous coronary angioplasty (PCI). The aim of the present study was to improve the antiplatelet regimen with novel drugs in desirable. Buccal patches for the delivery of clopidogrel bisulphate using HPMC (E5LV), pectin, sodium alginate with various hydrophilic polymers in various proportions and combinations were fabricated by solvent casting method. Various physicochemical parameters like weight variation, thickness, tensile strength, folding endurance, drug content, moisture uptake were studied. An *in-vitro* drug release study was designed, and it was carried out using cellophone membrane and *ex-vivo* study by goat membrane. All these fabricated patches for fast dissolving and obeyed first order release kinetics indicated that fickian type (case-1) diffusion mechanism.

INTRODUCTION

The novel bioadhesive mucosal dosage forms including adhesive tablets, gels, patches and more recently the use of polymeric films for oral cavity delivery, also known as mouth dissolving buccal patches gained attention in formulation research and growing popularly day by day in the global pharma industry[1]. Oral route has been the commonly adopted and most convenient route for drug delivery. This route has been received more attention in the pharmaceutical field because of the more flexibility in the designing of dosage form than drug delivery design for other routes, ease of administration as well as traditional belief that by oral administration the drug is well absorbed as the food stuffs that are ingested daily.

Antiplatelet or antithrombotic drug are the cornerstone in the modern therapy of patients with acute coronary syndrome (ACS), because of the unique role of platelets in coronary thrombosis. Clopidogrel is a thienopyridine which inhibits ADP-induced platelet aggregation, with no direct effects on the metabolism of arachidonic acid.

The other modes of drug delivery in to the body were investigated.

Those are,

1. Trans Dermal Drug Delivery System (through the intact skin)

2. Trans Mucosal Drug Delivery System (through the intact mucosa of the mouth, intestine, rectum, vagina or nose)

3. Trans Ocular Drug Delivery System (through the eye)

4. Trans Alveolar Drug Delivery System (inhalation through the lung tissue)

5. Implantable Drug Delivery System (through the subcutaneous and deeper implants, deliver into surrounding tissue)

6. Injectables (I.M or Subcutaneous) of the above modes, Transdermal, Transmucosal, Injectables and Subcutaneous Implants have been found varying degree of commercial acceptance[2].

An ideal buccal patch should be flexible, elastic and soft yet adequately strong to withstand breakage due to stress from mouth activities. Moreover, it must also exhibit good mucoadhesive strength so that it can be retained in the mouth for a desired duration. As such,

the mechanical, mucoadhesive, and swelling properties of buccal patches are critical and essential to be evaluated. The buccal route has high acceptance due to avoidance of 1st pass metabolism and possibility of being accessible for controlled drug release[3]. Table No. 1 shows the blood flow in the various regions of the oral mucosa.

Table No. 1: Blood flow in the various regions of the oral mucosa

S. No	Tissue	Blood flow (ml/ 100cm^2)
1.	Buccal	2.40
2.	Sublingual	3.14
3.	Floor of mouth	0.97
4.	Ventral tongue	1.17
5.	Frenulum	1.00
6.	Gingival (+)	1.47
7.	Palatal (-)	0.89

(+) Average value of maxillary and mandibular attached gingival mucosa.

(-) Average value of anterior and posterior hard palatal mucosa.

Oral mucosal sites

- **Sublingual delivery:** is the administration of the drug via the sublingual mucosa (the membrane of the ventral surface of the tongue and the floor of the mouth) to the systemic circulation.

- **Buccal delivery:** is the administration of drug via the buccal mucosa (the lining of the cheek) to the systemic circulation.

- **Local delivery:** for the treatment of conditions of the oral cavity, principally ulcers, fungal conditions and periodontal disease. These oral mucosal sites differ greatly from one another in terms of anatomy, permeability to an applied drug and their ability to retain a delivery system for a desired length of time[4,5].

Overview of oral mucosa

- The anatomical and physiological properties of oral mucosa had been extensively reviewed by several authors[4]. The oral cavity comprises the lips, cheek, tongue, hard palate,

soft palate and floor of the mouth. The lining of the oral cavity is referred to as the oral mucosa and includes the buccal, sublingual, gingival, palatal and labial mucosa.

- The buccal, sublingual and mucosal tissues at the ventral surface of the tongue accounts for about 60% of the oral mucosal surface area. The top quarter to one-third of the oral mucosa is made up of closely compacted epithelial cells. The primary function of the oral epithelium is to protect the underlying tissue against potential harmful agents in the oral environment and from fluid loss[6].

- Beneath the epithelium are the basement membrane, lamina propia and submucosa. The oral mucosa also contains many sensory receptors including the taste receptors of the tongue. Three types of oral mucosa can be found in the oral cavity; the lining mucosa is found in the outer oral vestibule (the buccal mucosa) and the sublingual region (floor of the mouth) as shown in Fig No. 1.

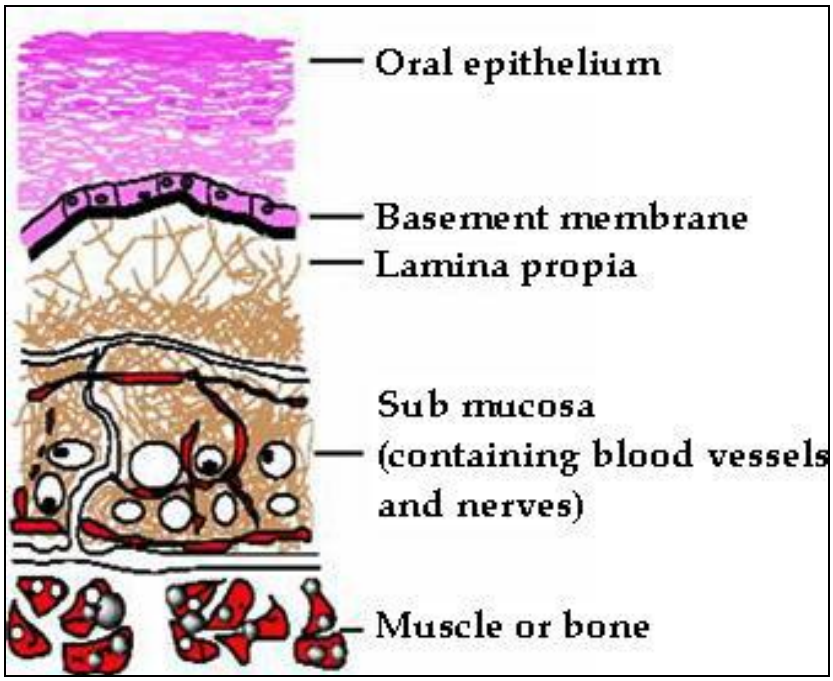

Figure No. 1: Schematic Diagram Showing the Principal Components of Oral Mucosa

Advantages[7]

- The oral mucosa has a rich blood supply. The buccal administration, the drug gains direct entry into the systemic circulation there by passing the first pass effect.

- It is richly vascularized and more accessible for administration and removal of dosage forms.
- No hepatic first-pass effect.
- No pre-systemic metabolism in the gastrointestinal tract.
- Ease of administration.
- High patient accessibility.
- An expanse of smooth muscle and relatively immobile mucosa, suitable for administration of retentive dosage forms.
- More rapid cellular recovery and achievement of a localized site on smooth surface of buccal mucosa.
- Low enzyme activity, suitability for drugs/ excipients that mildly and reversibly damages or irritates the mucosa.

Disadvantages[8]

- Low permeability of buccal membrane specifically when compared to the sublingual membrane.
- Small surface area (170 cm^2).
- Saliva (0.5–2 L/day) is continuously secreted into the oral cavity diluting drugs at the site of absorption resulting in low drug concentrations at the surface of the absorbing membrane. Inconvenience of patient when eating or drinking.

MATERIALS AND METHODS

Clopidogrel Bisulphate and HPMC (E5LV) was a gift sample from Vertex Pharma Chemicals, Pondicherry; pectin from Hi Media Laboratories, Mumbai; sodium alginate from Merck Limited, Mumbai and glycerine, DMSO from various commercial sources.

PREPARATION OF CLOPIDOGREL BISULPHATE BUCCAL PATCHES

By using Solvent Casting method the required quantity of drug with different polymers (HPMC-E5LV, pectin, sodium alginate) ratios are (1:4, 1:6, 1:8) was prepared. The

excipients were dissolved in water then polymer was dissolved and Clopidogrel Bisulphate drug was added. Solution was stirred with help of magnetic stirrer (Rotek, W. Vengola, Kerala, India) for 5mins to form a homogenous solution. Finally casted in to the petriplate and dried at 40ºC temperature in hot air oven.

Table No. 2: Formulation of Buccal patch of Clopidogrel Bisulphate

S. No	INGREDIENTS	HPMC (E5LV)			PECTIN			SODIUM ALGINATE		
		H1	H2	H3	P4	P5	P6	S7	S8	S9
1	Clopidogrel bisulphate (mg)	75	75	75	75	75	75	75	75	75
2	Polymer (mg)	300	450	600	300	450	600	300	450	600
3	DMSO (ml)	0.3	0.5	0.7	0.3	0.5	0.7	0.3	0.5	0.7
4	Glycerin (ml)	0.3	0.5	0.7	0.3	0.5	0.7	0.3	0.5	0.7
5	Water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

RESULTS AND DISCUSSION

Thickness of the Patch[9]

The thickness of the patch was assessed by using digital vernier caliper at different points of the patch. From each formulation three, randomly selected patches were used. The average value for thickness of a single patch was determined. The result was shown in Table No. 3.

Folding Endurance[10]

Folding endurance of the patches was determined by repeatedly folding one patch at the same place till it broke or folded up to 300 times manually, which was considered satisfactory to reveal good patch properties. The number of times the patch could be folded at the same place without breaking gives the value of the folding endurance. This test was done on five patches.

Percentage Moisture Content[11]

The patch were weighed and kept in desiccator containing calcium chloride. After 24 hrs the patch were taken out and weighed. The percentage moisture content was calculated using the following formula and the result was shown in Table No. 3.

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

Percentage moisture uptake[12]

The patch were weighed accurately and placed in desiccator containing aluminium chloride. After 24 hrs the patch were taken out and weighed. The percentage moisture uptake was calculated as the difference between final and initial weight with respect to initial weight it is calculated by using following formula. The result was tabulated in Table No. 3.

$$\text{Percentage moisture uptake} = \frac{\text{Finial weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Determination of Surface pH[13]

A combined glass electrode was used for this purpose. 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. Patches were placed in glass tubes containing 10ml PB (6.8). Placing the dip of the glass.

Tensile strength

Tensile strength is the maximum stress applied to a point at which the film specimen breaks. It is calculated by the applied load at rupture divided by the cross-sectional area of the strip as given in the equation below.

$$\text{Tensile strength} = \frac{\text{Load at failure}}{\text{Film thickness X Film width}} \times 100$$

Uniformity of weight

This was done by weighing five different patches of individual batch taking the uniform size of random and calculating the average weight of three. The tests were performed on strip which was dried at 60º C for 4 hrs prior to testing. The result was shown in Table No. 3.

Drug content determination[14]

The patch were taken and added to a beaker containing 100ml of phosphate buffer saline pH 6.8. The medium was stirred by magnetic bead for 60 mins. The solution was later filtered and analyzed for drug content with proper dilution at 202nm spectrophotometrically and the result was shown in Table No. 3.

Percentage elongation[15]

When stress is applied, a strip sample stretches and this is referred to as strain. Strain is basically the deformation of strip divided by original dimension of the sample. Generally, elongation of strip increases as the plasticizer content increases.

$$\text{Percentage elongation} = \frac{\text{Increase in length of strip}}{\text{Initial length of strip}} \times 100$$

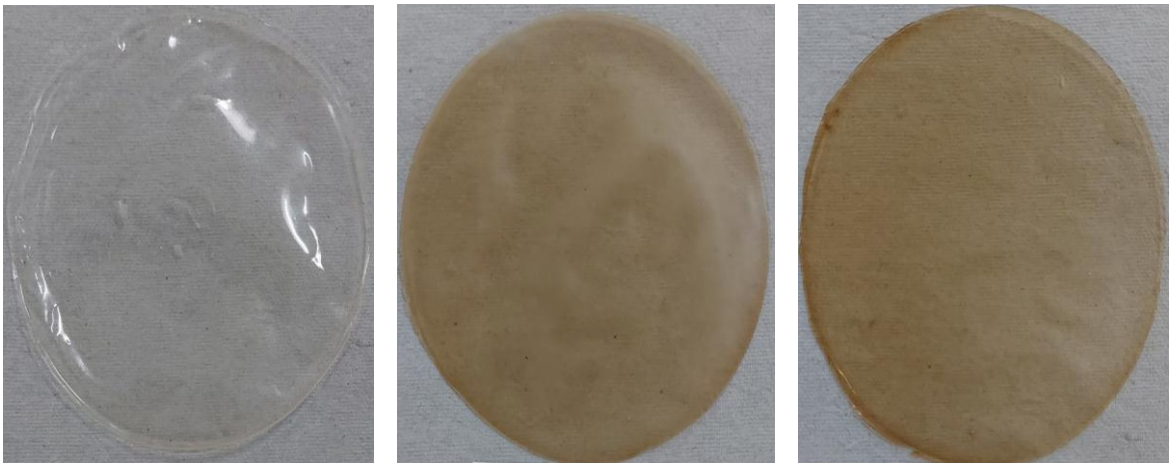

Figure No. 2, 3 & 4: Buccal patch of H2, P4, S8

Table No. 3: Physicochemical Evaluation of Clopidogrel Bisulphate Buccal Patch

Formulation code		Thickness (mm)	Folding endurance (no's)	Moisture content (%)	Moisture uptake (%)	Surface pH	Tensile strength (Kg/mm^2)	Uniformity of weight (g)	Drug content (%)	% Elongation (mm)
HPMC (E5LV)	H1	0.18± 0.54	265± 0.08	0.574± 0.62	2.08± 0.56	6.6± 0.07	3.412± 0.07	0.25± 0.75	94.49± 0.07	68± 0.54
	H2	**0.26± 0.49**	**269± 0.59**	**1.927± 0.6**	**2.16± 0.09**	**6.4± 0.12**	**5.402± 0.67**	**0.43± 0.3**	**98.84± 0.67**	**76± 0.45**
	H3	0.27± 0.63	275± 0.85	1.626± 0.07	1.99± 1.05	6.3± 0.25	5.463± 0.85	0.39± 0.47	95.73± 0.70	80± 1.23
Pectin	**P4**	**0.19± 0.17**	**258± 0.60**	**1.273± 0.6**	**2.18± 0.09**	**7.0± 0.13**	**3.162± 0.23**	**0.39± 0.03**	**95.26± 0.67**	**75± 0.23**
	P5	0.20± 0.85	260± 0.43	1.069± 0.05	2.09± 0.69	6.8.± 0.45	4.563± 0.48	0.28± 0.23	94.09± 0.31	83± 0.74
	P6	0.24± 0.61	265± 0.69	1.133± 0.64	2.07± 0.36	6.9± 0.32	5.412± 0.74	0.29± 0.27	93.95± 0.83	85± 0.94
Sodium alginate	S7	0.13± 0.53	260± 0.42	1.483± 0.47	0.98± 0.43	6.7± 0.9	3.802± 0.22	0.27± 0.4	94.02± 1.06	80± 0.92
	S8	**0.19± 0.34**	**265± 1.5**	**1.194± 0.10**	**1.90± 0.21**	**6.2± 0.32**	**4.662± 0.91**	**0.33± 0.63**	**97.02± 0.49**	**88± 0.08**
	S9	0.21± 0.47	272± 1.0	1.185± 0.03	1.91± 0.04	6.8± 0.29	5.410± 1.65	0.30± 1.28	96.78± 0.72	90± 0.81

Mean ±S.D: n = 3

Based on the thickness, folding endurance, moisture content, moisture uptake, surface pH, tensile strength, uniformity of weight, drug content and elongation the formulation H2, P4, S8 were selected for further studies.

IN-VITRO DRUG RELEASE

The *in-vitro* release rate of Clopidogrel Bisulphate buccal film were evaluated by open ended tube through using phosphate buffer solution pH 6.8 as diffusion medium up to 60 seconds studies. The cellophone membrane is tied in one end of the tube and then immersed in the receptor compartment containing 400ml of PBS pH 6.8 which was stirred at medium speed and maintained at 37ºC ± 2ºC. Samples were withdrawn at regular time intervals and the same volume was replaced by fresh diffusion medium. The samples were analyzed using UV double beam spectrophotometer set at 203nm.

Table No. 4: *In vitro* drug release of H2, P4, S8

Time (sec)	% of drug released		
	H2	P4	S8
0	0	0	0
10	14.06 ± 0.21	9.92 ± 0.37	12.08 ± 0.34
20	28.15 ± 0.02	25.74 ± 0.45	29.00 ± 0.05
30	45.70 ± 0.21	41.11 ± 0.56	40.90 ± 0.07
40	69.99 ± 0.12	66.95 ± 0.40	67.03 ± 0.59
50	67.49 ± 0.05	85.93 ± 0.04	83.00 ± 0.38
60	98.86 ± 0.07	95.67 ± 0.09	94.89 ± 0.40

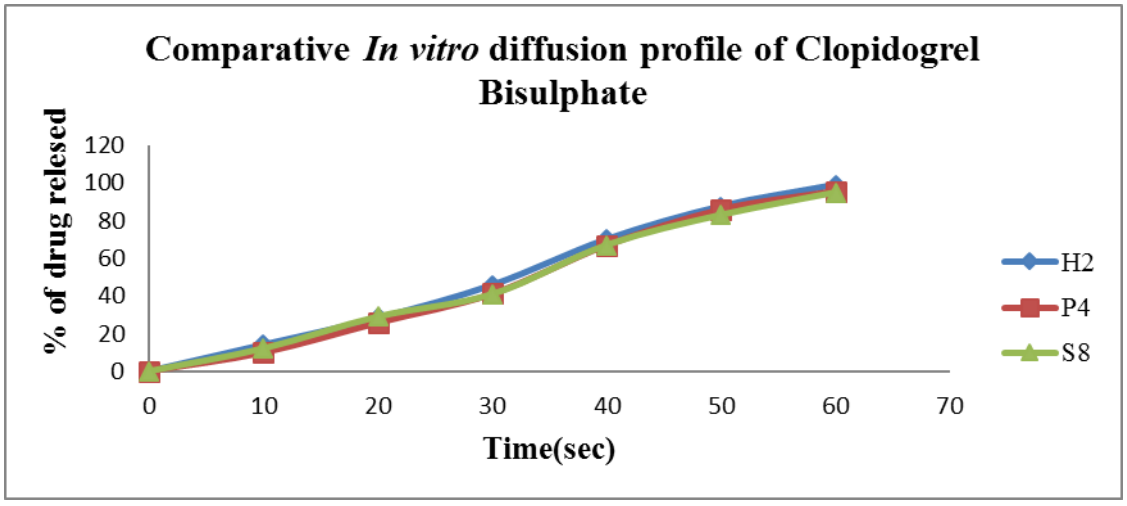

Figure No. 5: *In vitro* drug release of H2, P4, S8

FT – IR ANALYSIS

Infrared spectrum of any compound or drug gives information about the groups present in that particular compound. The absorption spectra of the pure drug and physical mixtures of drug with various excipients were taken in the range of 4000- 400 cm^{-1} using KBr disc method (Schimadzu IR- prestige - 21) and observed for characteristic peak of drug shown below in Fig No. 6 & and Fig No. 7.

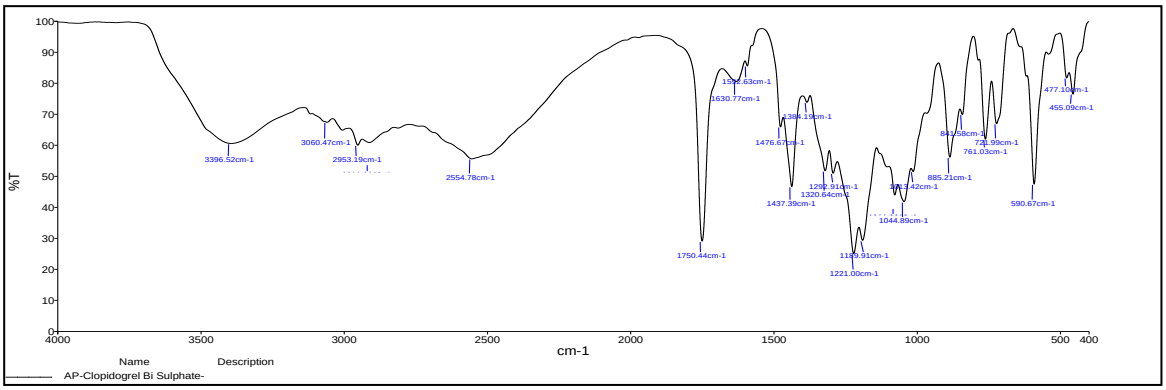

Figure No. 6: FT-IR Spectrum of CB

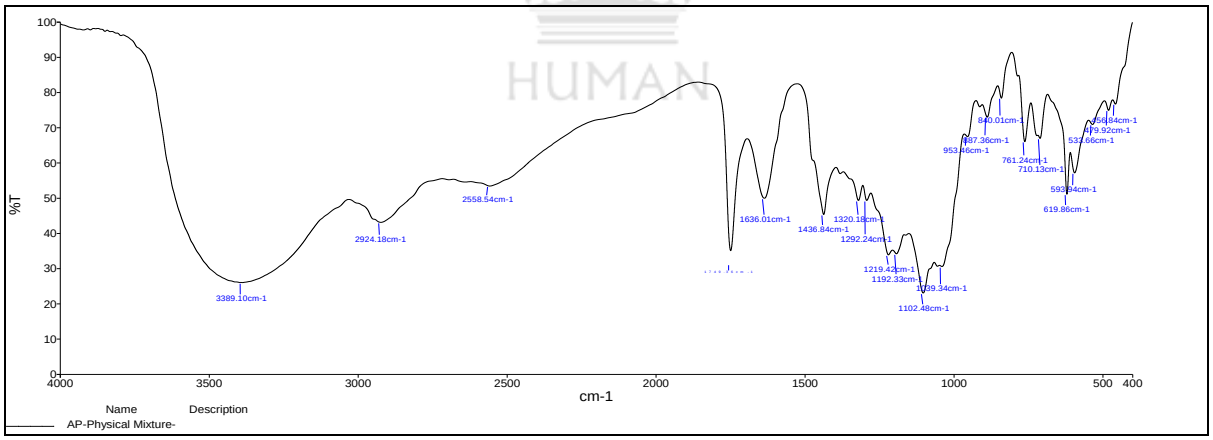

Figure No. 7: FT-IR Spectrum of Mixture of Polymers

Observation- Drug – excipient compatability was carried out by FTIR analysis. Initially, the FTIR spectrums of Clopidogrel Bisulphate, HPMC (E5LV), Pectin, Sodium alginate, DMSO, Glycerin were obtained. After that, the mixtures of drug with excipients were prepared and FTIR spectrum was obtained. The obtained spectra of physical mixtures was observed for major peaks and recorded. The results of this observation were concluded that there is no interaction between the drug and other excipients.

RELEASE KINETICS

Data obtained from *in-vitro* release studies were fitted to various kinetic equations. The kinetic models used are zero order equations (Q=K0t), First order equation (In (100 - Q) =In Q – KIt), Higuchi equation (Q = KtI/2), Hixon and crowell model Qt1/3 Vs and Qt2/3 Vs – Modified root cube equation. Further, to find out the mechanism of drug release, first 60% drug release was fitted in Korsmeyer and Peppas equation (Q = Kptn). Where, Q is the percent of the drug release at time t and K0 and Kt are the coefficients of the equations and 'n' are the release exponent. The n- value is used to characterize different release mechanism are tabulated below.

Table No. 5: Release kinetics of H2, P4, S8

Kinetic Models	Statistical parameters	Formulation H2	Formulation P4	Formulation S8
Zero order	R^2	0.992	0.986	0.990
First order	R^2	0.864	0.864	0.864
Higuchi	R^2	0.882	0.878	0.872
Korsemeyer peppas	R^2	0.995	0.993	0.993
	n	1.163	1.163	1.163

Observation - The release kinetics showed that the H2 formulation followed first order and drug release follow no fickian diffusion mechanism.

EX-VIVO STUDY

Ex-vivo buccal permeation studies carried out using Goat buccal membrane. The receptor compartment consisted of 400ml of Phosphate buffer (pH 6.8) in 500ml beaker. Temperature was maintained at 37±0.5ºC and stirred at 900rpm. The cineole strip was placed in Goat buccal membrane and tied to the one end of open-ended glass cylinder that was then dipped into freshly prepared phosphate buffer on magnetic stirrer. Samples were taken from receptor medium at 0, 10, 20, 30, 40, 50, 60 sec. Periodically 5ml of sample was withdrawn and some volume of medium was replaced with fresh buffer to maintain sink condition. All the samples were analyzed spectrophotometrically at 203nm using PB 6.8 pH as blank. The results shown in Table no. 6 & Fig. no. 6.

Table No. 6: *Ex-Vivo* Study of H2 Optimized Formulation

Time (sec)	% of drug diffused
	H2 formulation
0	14.02 ± 0.03
10	28.13 ± 0.28
20	45.67 ± 0.01
30	69.29 ± 0.87
40	87.40 ± 0.40
50	98.69 ±0.05

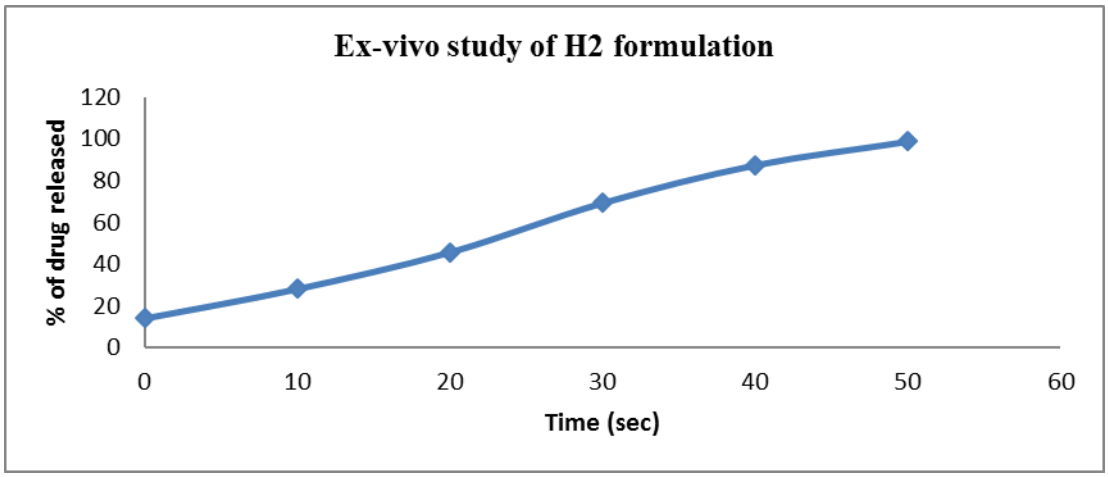

Figure No. 8: *Ex-Vivo* Study of H2 Optimized Formulation

STABILITY STUDY

The stability studies of optimized (H2) formulation of buccal film were carried out for 3 months. During this period, the formulation was stable and showed no significant changes in visual appearance, colour, texture and drug content.

ACKNOWLEDGEMENT

The authors would like to thank Vertex Pharma Chemicals, Pondicherry, for priority Clopidogrel Bisulphate as a gift sample.

CONCLUSION

Nine batches of Clopidogrel Bisulphate buccal patches were prepared by using three different polymers (HPMC-E5LV, Pectin, Sodium alginate) in different Drug-Polymer ratio (1:4, 1:6, 1:8). From the various formulations, the patch were optimized to get thin, transparent, smooth, stable and high permeable transdermal patches. Based on the physicochemical parameters such as appearance, thickness, tensile strength, uniformity of drug content, and *in vitro* diffusion studies H2, P4 and S8 were selected as best 97.78% of drug was released at the end of 60sec for H2 formulation. The data obtained from *in vitro* release profile was fitted with various kinetic equations to determine the mechanism of drug release rate as indicated by higher regression correlation coefficient (r^2). From the release kinetic results, the r^2 value of H2 was found to be higher in first order release kinetics. In case of korsmeyer peppas model, the result indicated that fickian type (Case-1) diffusion mechanism. From the results, it may concluded that the buccal patches H2 containing HPMC(E5LV) in the ratio 1:4 achieved the objectives of quick release (within 60 sec) and accurate dosing (97.78%). Thus, the present study deliver the drug constantly and slowly demonstrated potentials for rapid absorption can be effective therapy and patient compliance for the treatment of thrombosis.

ABBREVIATION

ACS- Acute Coronary Syndrome

ADP- Adenosine Di Phosphate

CB - Clopidogrel Bisulphate

DMSO – Dimethyl Sulfoxide

NSTEMI – Non ST-Segment elevation myocardial infarction

IM –Intramuscular, PBS - Phosphate Buffer Solution

REFERENCES

1. Arun A, Amrish C, Vijay S, Kamala P. Fast Dissolving Oral Films; An Innovative Drug Delivery System and Dosage Form. *Int J of Chem Tech Research*, 2010; 2(1): 576-583.
2. Donald L. Wise, Handbook of pharmaceutical controlled release technology, pp.no. 431-433.
3. Khanna R, Agarwal SP, Ahuja A. *Ind J Pharm Sci*, 1997; 59: 299-305, Ebert CD, Heiber SJ, Dave SC, Kim SW, Mix D. *J Control Release*, 1994; 28: 37-44.
4. Shojaei Amir H, Buccal Mucosa as A Route for Systemic Drug Delivery: A Review; *J Pharm Pharmaceut Sci* (www.ualberta.ca/~csps) 1998;1 (1):15-30. [View in PubMed].

5. Sevda Senel, Mary Kremer, Katalin Nagy and Christopher Squier, Delivery of Bioactive Peptides and Proteins Across Oral (Buccal) Mucosa, Current Pharmaceutical Biotechnology, 2001; 2: 175-186.
6. Dowty ME, Knuth KE, Irons BK, Robinson JR. Transport of thyrotropin releasing hormone in rabbit buccal mucosa *in vitro*. *Pharm Res*. 1992;9: 1113-22.
7. Andrews Gavin P, Mucoadhesive polymeric platforms for controlled drug delivery, *Eur J Pharm Biopharm* 2009; 71:505-518.
8. Y.Madhusudan Rao, A.V. Jithan, Advances in drug delivery, Volume-1, pp.no.139-198.
9. Nazila Salamat – Miller, Montakarn Chittchang, Thomas P. Johnston. The use of mucoadhesive polymers in buccal drug delivery. *Advanced Drug Delivery Reviews* 57 (2005) 1666-1691.
10. Apoorva Mahajan, Formulation and evaluation of overview of buccal film, used in different polymer, 2013.
11. Javier O. Morales, Jason T. Mc Conville. Manufacture and characterization of mucoadhesive buccal films. European Journal of Pharmaceutics and biopharmaceutics 77(2011) 187-199.
12. Narasimha Rao R, Sindhu Reddy Kandhadl, Swapna D, Sushma Devi Konasree, Swathi Enugala. Formulation and evaluation of rapidly dissolving buccal patches. IJPBS/Volume I/ Issue 3/July-Sept/2011/145-159.
13. Semalty M, Semalty A, Kumar G. Formulation and characterization of mucoadhesive buccal films of glipizide. *Ind J Pharm Sci*. 2008; 70(1): 43 – 48.
14. Nina Langoth, Jochen Kalbe, Andreas Bernkop – Schnurch. Development of buccal drug delivery systems based on a thiolated polymer. *International Journal of Pharmaceutics* 252: 2003, 141 – 148.
15. Doijad RC, Manvi FV, Rao VSNM, Patel PS. Buccoadhesive drug delivery system of Isosorbide Dinitrate: formulation and evaluation. Ind J Pharm Sci. 2006;68(6):744-748.