WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 8, 1521-1533. Research Article ISSN 2277– 7105

DEVELOPMENT AND EVALUATION OF MUCOADHESIVE BUCCAL PATCH

Shrutika Zile*[1] , Vaishali Wasnik*[2], Shilpa Gawande[1] , Bharti Bakde[1], Madhuri Channawar[1]

[1]Department of Pharmaceutics, Pataldhamal Wadhwani College of Pharmacy, Yavatmal, Maharashtra, India –445001.

[2]Shree Babasaheb Gharfalkar College of Pharmacy, Pulgaon, Maharashtra, India-442302.

Article Received on
29 May 2017,
Revised on 19 June 2017,
Accepted on 10 July 2017
DOI: 10.20959/wjpr20178-9024

***Corresponding Author**
Vaishali Wasnik Shree Babasaheb Gharfalkar college of Pharmacy, Pulgaon-442302.

ABSTRACT

The present investigation involves formulation and evaluation of buccal patch of Valsartan, an antihypertensive drug to bypass the first pass metabolism. The buccal patch of Valsartan was prepared by solvent casting technique. Nine formulation were prepared with varying concentrations of bioadhesive polymers like Hydroxy propyl methyl cellulose K-100M and Sodium Alginate. Solvent ethanol was used with PEG-200 as plasticizer and Aspartame as sweetening agent. Drug-polymer interaction was investigated using FTIR. The prepared patches were evaluated for thickness, Surface pH, drug content uniformity, Ex vivo Mucoadhesive strength, % swelling index, folding endurance and in vitro drug release, Determination of Residence Time, Ex Vivo Permeation Study which produced satisfactory results with low standard deviation. After evaluation of all parameter, on the basis of results obtained batch F8 was found to be a optimize batch. This batch shows 97.15% Controlled Drug Release after 8 hrs and best fitted in Higuchi model for drug release kinetic.

KEYWORDS: Mucoadhesive, Valsartan, Drug delivery, Controlled Drug Release.

INTRODUCTION

The oral cavity is an alternative site for Drug delivery due to ease of administration and avoidance of possible drug degradation in the GIT, and first pass metabolism. Buccal delivery drugs may be used for treatment of diseases in the oral cavity (or) for systemic use.[1] However inherent limitation including short residence time, small absorption area and barrier property of the buccal mucosa is challenges to buccal drug delivery. Additionally, buccal

drug delivery has a high patient acceptability compared to other non-oral route of drug administration. Moreover, rapid cellular recovery and achievement of a localized site on the smooth surface of the buccal mucosa are among the other advantages of this route of drug delivery.[2]

In present study, an attempt was made to formulate and evaluate mucoadhesive buccal patches of Valsartan having low bioavailability due to extensive hepatic or GI metabolism. Valsartan is angiotensin ll receptor antagonist. Therapeutically Valsartan is advised in hypertension. It is practically insoluble in water. It is absorbed from GIT but show only 25% bioavailability due to extensive hepatic metabolism.[3] It has been suggested that drugs with biological half life lives in the range of 2-8 hours are good candidate for sustained release formulation.[4] Total nine formulation were prepared by using HPMC K 100M in combination with sodium alginate in various ratios and compare all the formulations by their physicochemical parameters and drug release pattern.

MATERIALS AND METHODS

Materials

Valsartan was supplied as a gift sample by Macleods Pharmaceutical Limited, Mumbai. Hydroxy propyl methyl cellulose-K-100M procured from Colorcon Asia Pvt. Limited, Goa. Sodium Alginate and Aspartame procured from ACME Chemicals, Mumbai, Polyvinyl Alcohol, PEG-200 were purchased from MCC Laboratory Chemicals. Ethanol was purchased from Yarrow Chem Product, Mumbai.

Fabrication of Medicated Patches

Buccal patches of Valsartan were prepared by solvent casting technique, using combination of two polymers sodium alginate and Hydroxy propyl methyl cellulose K-100m The weighed amount of drug Valsartan was dissolve in 2ml of solvent ethanol with slow stirring for 5 min. sodium alginate and HPMC-K-100M was dissolved in ethanol with continuous stirring on magnetic stirrer for 2 hr. Then both solutions were mixed together with slow stirring to get a clear viscous solution. Polyethylene Glycol 200 was used as plasticizer. The complete formula is shown in Table 1. The solution was poured in a petridish and allowed to dry over night at room temperature to remove the bubbles. Then solution was dried in an oven maintained at 40ºC till a flexible patch was formed. The dried patch was carefully removed from the petridish and cut into squares of 1.5 cm^2.

Backing membrane[8]

PVA is used as a backing membrane for unidirectional drug release.400 mg of PVA was dissolve in 10 ml of ethanol with continuous stirring on magnetic stirrer for 2 hr.0.2 ml of PEG-200 was used as plasticizer. The solution was poured in a petridish and allowed to dry over night at room temperature to remove the bubbles. Then solution was dried in an oven maintained at 40ºC till a flexible patch was formed. The dried patchwas carefully removed from the petridish and cut into squares of 1.5 cm^2.

Evaluation of patches

Weight Uniformity[8]

Three strips of same size (1 x 1.5 cm) of each formulation were weighed individually on a electronic balance and the average weight was calculated.

Thickness Study[8]

The thickness of the patch was measured using screw gauge with a least count of 0.01mm at different spots of the patches. The thickness was measured at three different spots of the patch from every batch and average thickness was calculated.

Surface pH[5,8]

The surface pH of the patch was determined by the method similar to that used by Bottenberg et al. (1991). The patches were allowed to swell by keeping them in contact with 1drop of distilled water for 2h at room temperature and pH was noted down by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1 min.

Content Uniformity[5,8,9]

Drug content uniformity was determined by dissolving the patch (dimension 1 x 1.5 cm) by homogenization in 50 ml of an isotonic phosphate buffer pH 6.8 for 2 hr with occasional shaking. Aliquot 1 ml was withdrawn and diluted with isotonic phosphate buffer pH 6.8 up to 10ml and the resulting solution was filtered through a 0.45mm Whatman filter paper. The drug content was then determined after appropriate dilution by using UV spectrophotometer at 250nm.

Swelling studies[5-9]

The degree of swelling of bioadhesive polymer is an important factor affecting adhesion. The swelling rate of buccoadhesive patch was evaluated by placing the patch in phosphate buffer

solution pH 6.8 at 37 ± 1ºC. The patches of each batch were cut and weighed (W1). The patches were placed in phosphate buffer and were removed at time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hr. Excess water on the surface was carefully absorbed using filter paper and swollen patches were reweighed. The average weight W2 was calculated and the swelling index was calculated by the formula:

Swelling index = [(W2 -W1) ÷ W1] × 100 Where, W1 = Initial weight of the patch

W2 =Final weight of the patch

Folding endurance[5-9]

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

Ex vivo Mucoadhesivestrength[5-7]

Buccoadhesive strength of the buccal patches was measured on the―Modified Physical Balance method‖. The method used fresh sheep buccal mucosa as the model mucosal membrane. The fresh sheep buccal mucosa was cut into pieces and washed with phosphate buffer pH 6.8. A piece of mucosa was stuck on glass stopper of uniform surface which was moistened with phosphate buffer pH 6.8. The patch was stuck to the lower side of another glass slide with glue. Then both pans of the balance were balanced by adding an appropriate weight on the left hand pan. The metal plate with mucosa was placed with appropriate support, so that the patch touches the mucosa. Previously weighed beaker with water was placed on the right hand pan and water (equivalent to weight) was added slowly to it until the patch detached from the mucosa surface. The weight required to detach the patch from the mucosal surface gave the buccoadhesive strength.

Method

The balance adjusted as described above was used for the study. The sheep buccal mucosa, excised and washed was tied tightly with mucosal side upward using thread over the base of inverted 50 ml glass beaker. This beaker suitably weighted was lowered into 500ml beaker, which was then filled with isotonic phosphate buffer (pH 6.8) kept at 37ºC such that the buffer reaches the surface of mucosal membrane and keeps it moist. This was then kept below left hand side of balance. The buccal patch was then stuck to glass stopper through its

backing membrane using an adhesive (acrylic glue, Feviquick). The 5gm on right hand side is removed; this causes application of 5gms of pressure on buccal patch overlying moist mucosa. The balance was kept in this position for 3 minutes and then slowly weights were increased on the right pan, till patch separates from mucosal membrane. The total weight on right pan minus 5gms gives the force required to separate patch from mucosa. This gives bio-adhesive strength in grams. The mean value of three trials was taken for each set of formulations. After each measurement, the tissue was gently and thoroughly washed with isotonic phosphate buffer and left for 5 minutes before reading. A new patch of same formulation is used for each reading to get reproducible multiple results for the formulation.

The following parameter were calculated from the mucoadhesive strength:

$$\text{Force of adhesion(N)} = \frac{\text{Mucoadhesive strength (g)} \times 9.81}{1000}$$

Determination of Residence Time[7-9]

The in vitro residence time was determined using a locally modified USP disintegration apparatus, based on the apparatus applied by Nakamura et al 20. The disintegration medium was composed of 800 ml pH 6.8 isotonic phosphate buffer (IPB) maintained at 37 ± 0.5°C. A porcine buccal mucosa, 3cm length, was glued to the surface of a glass slab, vertically attached to the apparatus. The mucoadhesive patch was hydrated from one surface using 15µl pH 6.6 IPB and then the hydrated surface was brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down so that the patch was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time necessary for complete erosion or detachment of the patch of each batch from the mucosal surface was recorded in Table 5.

In Vitro Drug Release[5-7]

The USP dissolution apparatus type 2(rotating paddle method) was used to study the drug release from buccal patches. The dissolution medium consisted of 900ml of isotonic phosphate buffer pH 6.8. The release was performed at 37 ± 0.5°C, at a rotation speed of 50rpm. One side of the buccal patch was attached to a glass disk with instant adhesive (cyanoacrylate). The disk was put in the bottom of the dissolution vessel so that the patch remained on the upper side of the disk. Samples (5ml) were withdrawn by using calibrated pipette at pre-determined time (1hour) intervals and replaced with fresh medium. The samples were filtered through 0.45µm Whatman filter paper with appropriate dilutions with

phosphate buffer pH 6.8 and were assayed by UV spectrophotometrically at 250nm.

Ex Vivo Permeation Study[5,7]

The buccal permeation test by using sheep buccal mucosa was planned for optimized batch only. Diffusion study of further batches was carried out by using Artificial Dialysis membrane as barrier membrane.

In this study, porcine buccal mucosa was used as a barrier membrane. Diffusion studies were carried out, to evaluate the permeability of drug across the porcine buccal mucosal membrane, by using glass surface Franz diffusion cell. Porcine buccal mucosa was obtained from local slaughter house and used within 2 hrs. The tissue was stored in phosphate buffer pH 6.8 solution upon collection. The epithelium was separated from underlying connective tissues with surgical scissors clamped between donor and receiver chamber of diffusion cells for permeation studies. The smooth surface of the mucosal membrane faced the donor chamber and receiver chamber was filled with phosphate buffer of pH6.8. Whole assembly was placed on a magnetic stirrer maintained at 37 ± 1^{0}C. Buccal epithelium was allowed to stabilize for 1hr and receiver chamber was maintained by stirring with magnetic bead at 50 rpm. After the stabilization of buccal epithelium, the patch was kept on buccal epithelium and 3ml of phosphate buffer pH 6.8 was added in donor chamber. Then samples of 4 ml were withdrawn at time intervals of 1 hour up to 8 hrs and replaced with equal volume of fresh dissolution medium. Sink condition was maintained throughout the study. The withdrawn samples were diluted to 10 ml. The amount of Valsartan was determined by UV-VIS Spectrophotometer at 250 nm. The drug permeation was correlated with cumulative drug released.

RESULTS AND DISCUSSION

Prior to the formulation, detection of melting point and solubility was carried out on drug. A buccal patches containing valsartan was prepared using combination of polymer sodium alginate and HPMC K 100m. In the formulation, ethanol issued as casting solvent. The drug was dissolved polymeric solution containing PEG-200 as plasticizer. The detailed parameter like weight uniformity, thickness of patch, surface pH, in vitro residence time, ex vivo permeation study, in vitro release study, mucoadhesive strength, stability study and release kinetic study.

Melting point

The average of three determination of melting point was carried out and the result is found to be 116-117^{0}C. The melting point was found in the range of standard reading.

Solubility

The drug Valsartan is practically insoluble in water. Soluble in methanol and ethanol**.**

Infra-Red Spectroscopy

An IR spectral study was performed for batch F8. It is concluded that the drug valsartan shows compatibility with the polymer sodium alginate and HPMC K 100m.

EVALUATION OF BUCCAL PATCHES

Appearance

The patches from all the batches were translucent and flexible without any sign of crack.

Weight Uniformity

The average weight of patch reported in table 16 and calculated by using three patches of sizes 1.5 cm^2. The weight of buccal patches ranges from 38 mg – 80 mg. The maximum average weight shown by batch F9 due to high concentration of polymer. The minimum weight shown by the batch F1 due to low polymer concentration.

Thickness Study

The average thickness of patch was reported in table 16 and calculated by using three patches of sizes 1.5 cm^2.The thickness of formulated patches ranges from 29 mm- 43 mm. The maximum average thickness shown by the batch F8 and F9. The minimum average thickness shown by the batch F1. This result occurs due to the high and low polymer concentration respectively.

Surface pH Study

The surface pH of patches reported in Table 3 and calculated by using three patches of sizes 1.5 cm^2. The pH of formulated patches ranges from 6.41-6.81.

Folding Endurance

The folding endurance of the buccal patches of all formulated batches was measured manually and it did not show any cracks even after folding at once place for more than 300 times to all formulated batches.

Drug Content

The drug content of patches reported in Table 3.

Swelling Study

The swelling percentage for batches F1-F9 was determined by measuring increased weight due to swelling after predetermined time. The swelling state of the polymer was reported to be crucial for its bioadhesive behavior. It was observed that the when the concentration of polymer HPMC K 100m and sodium alginate increases the % swelling is also increases.

Mucoadhesive Strength Measurement

Mucoadhesive strength of patches may be due to chemical bonding or it could be because of physical entanglement of swelled polymer with mucin thereby producing stronger mucoadhesion. The mucoadhesive strength of the formulated buccal patches on sheep buccal mucosa is due to sodium Alginate, HPMC-K 100 m in various ratios. As the concentration of buccal polymer increases the mucoadhesive strength of buccal patch is also increases. The data for batch F1-F9 is reported in Table 4.

Determination of In vitro residence time

The in vitro residence time of the formulated buccal patches on sheep buccal mucosa is due to the function of sodium alginate and HPMC- K 100m in various ratio. Residence time of optimized batch is only determined by using sheep buccal mucosa. The data of residence time for batch F1-F9 is reported in Table 5.

In vitro release study

The data for in vitro release study of Valsartan buccal patch of batches F1-F9 shown in Table 5. The in vitro release profile of Valsartan buccal patch of batches F7-F9 shown in Fig 1.

Ex vivo Permeation Study

The Ex- vivo buccal permeation study of batch F1-F9 was carried out to evaluate permeability of Valsartan across the buccal mucosal membrane. The permeation data for Valsartan shown in Table 7 and Fig 2.

Stability study

After duration of one month, the optimized batch F8 was subjected to % cumulative drug release, mucoadhesive strength, % swelling, drug content, thickness, surface pH, in vitro residence time and folding endurance. This study was carried out at accelerated condition of

40^0C ± 2 ^{0}C and 75 % ± 5 % RH, for a period of 1 month. The methods adopted were same as described earlier. The Sability study data for Valsartan shown in Table 8.

Stability in Human Saliva[32]

The stability of buccoadhesive patch was performed in natural human saliva using the optimized patch (F8) selected on the basis of swelling, bioadhesion, drug release and drug permeation. Human saliva was collected from healthy volunteers free from any disease. Patch were placed in separate Petri dishes containing 5ml of human saliva and kept in a temperature controlled incubator for 8hr at 37±0.2ºC. At regular time intervals (0, 1, 2, 3and 8hr), the patch were examined for changes in color. The data shown in Table 9.

Tables

Table 1: Composition of mucoadhesivebuccal patch of Valsartan

BATCH	Valsartan (mg)	HPMC-K 100 (mg)	Sodium Alginate (mg)	PEG-200 (ml)	Aspartame (mg)	Ethanol (ml)
F1	550	100	_	0.5	10	10
F2	550	150	_	0.5	10	10
F3	550	50	50	0.5	10	10
F4	550	100	50	0.5	10	10
F5	550	250	50	0.5	10	10
F6	550	250	100	0.5	10	15
F7	550	300	50	0.5	10	15
F8	550	300	100	0.5	10	15
F9	550	250	150	0.5	10	15

Table2: Peaks observed in mixture of Valsartan, sod.alginate, HPMC K 100M

Sr no.	Functional group	Peaks observed in drug (cm^{-1})	Peaks observed in poloymer (cm^{-1})
1	O ‖ --C—OH	3600-2750	3600-2750
2	CH^2 Aliphatic	2970	----
3	O ‖ --C---	1750	----
4	C=C Benzene	1600	-----
5	C—H Aromatic	3028	-----
6	C—H Aliphatic	2970	2970
7	C--O	--------	1100
8	C=O	1700	1700
9	C=C Bezene	1620	1620
10	N-H	3446	------

Table 3: Physicochemical characteristics of buccal patches of Valsartan

BATCH CODE	Weight Uniformity (mg)	Thickness Study (mm)	Surface pH study	Folding Endurance	Drug Content (%)
F1	38	0.25	6.73	>300	89
F2	40	0.26	6.81	>300	88
F3	71	0,35	6,64	>300	92
F4	50	0.26	6.70	>300	85
F5	52	0.25	6.52	>300	87
F6	61	0.32	6.79	>300	93
F7	70	0.42	6.80	>300	92
F8	73	0.43	6.77	>300	97
F9	81	0.43	6.72	>300	94

Table 4: Bioadhesive parameters for batch F1-F9

BATCH NO.	Mucoadhesive strength(gm)	Force of Adhesion(N)	Bond Strenght(Nm^{-2})
F1	33	0.323	0.215
F2	37	0.362	0.241
F3	30	0.294	0.196
F4	42	0.412	0.274
F5	45	0.441	0.294
F6	48	0.470	0.313
F7	53	0.519	0.346
F8	60	0.588	0.392
F9	51	0.500	0.333

Table 5: In vitro residence time for batch F1-F9

BATCH NO.	Ex vivo residence time (min)
F1	400
F2	425
F3	380
F4	435
F5	450
F6	485
F7	505
F8	520
F9	470

Table 6: Cumulative % Drug Release Data for batch F1-F9

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	6.780	5.975	35.15	45.25	51.24	54.03	54.05	40.56	53.60
2	6.905	7.173	60.76	63.13	68.90	56.61	78.65	58.92	55.71
3	8.842	8.393	61.78	65.41	72.56	58.81	80.13	66.07	60.06
4	12.850	11.704	62.98	67.60	74.33	80.36	81.31	73.09	73.01
5	15.538	12.704	64.36	68.37	77.05	81.56	81.97	79.61	75.60

6	22.66	16.651	67.30	69.91	78.46	81.80	99.03	89.03	87.09
7	30.33	33.11	70.82	71.07	80.13	89.13	103.5	93.05	99.01
8	42.30	30.15	72.80	76.09	87.30	90.13	104.1	97.15	101.01

Table 7: Ex vivo permeation study of batch F1-F9

Time (hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	0.54	0.28	1.88	1.65	1.2	5.16	1.14	7.07	5.03
2	1.99	1.12	2.47	2.95	1.9	8.85	3.57	16.88	6.50
3	7.59	3.54	7.69	6.54	3.84	14.82	5.54	22.43	9.39
4	10.35	6.36	8.45	13.24	7.65	15.64	13.14	24.43	16.98
5	12.35	10.79	21.95	21.54	16.24	18.65	13.61	39.11	28.37
6	26.38	12.85	22.9	28.24	22.34	26.32	31.07	62.58	28.76
7	32.87	26.35	34.84	29.84	34.85	46.24	40.58	74.58	43.22
8	39.24	31.58	40.38	36.14	55.24	85.59	82.03	97.33	86.66

Table 8: Stability data for optimize batch F8

Parameter	Zero month	One month
mucoadhesive strength	60 gm	59.55 gm
% swelling	65.60	65.18
drug content	97	95.86
thickness	0.44 mm	0.44 mm
surface pH	6.77	6.75
In vitro residence time	520 min	517min
folding endurance	>300	>300

Table 9: Stability in Human Saliva for batch F8

Sampling Time (hrs)	Observations
0	No change
1	No change
2	No change
3	No change
6	No change
8	No change

Figures

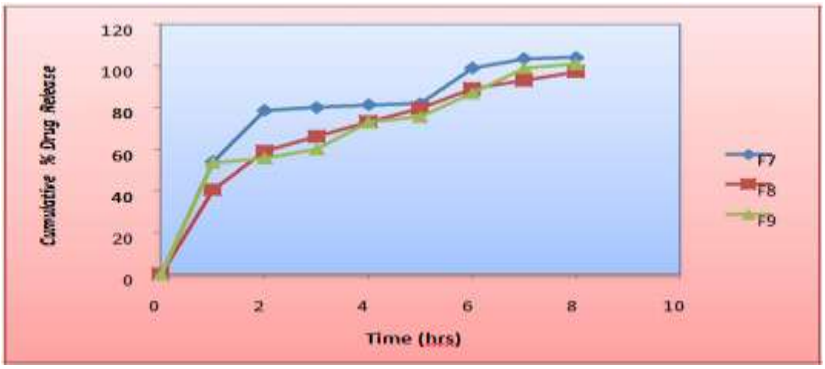

Fig 1: Cumulative % Drug Release Data for batch F7-F9

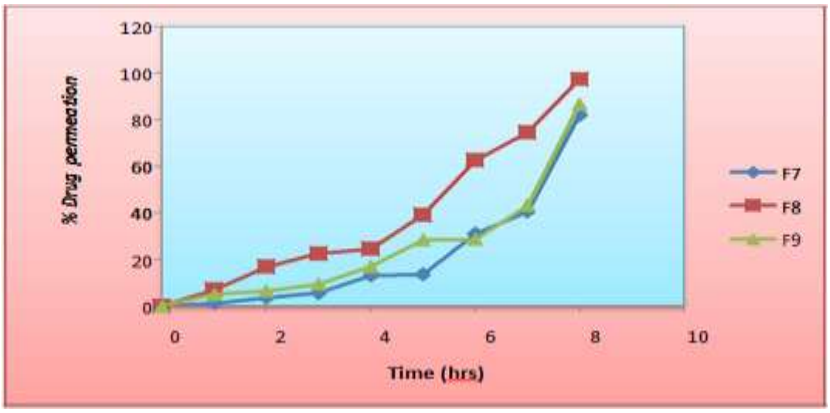

Fig 2: % Drug permeability from batch F7-F9

CONCLUSION

Metabolism of drugs during the passage through portal circulation before reaching the systemic circulation is a major concern in bioavailability. The present study demonstrates the successful development of buccal delivery system of Valsartan using sodium alginate and HPMC K 100M and it's in vitro evaluation. It depicts a practical formulation approach to improve the systemic availability of Valsartan or any drug suffering pre-systemic metabolism in the gut wall and liver. It was observed that drug release extended to 8 hours indicating the ability to serve as a once daily dosage form. In vivo study shall be conducted in future to assess the improvement in bioavailability of Valsartan. This article provide a valuable information regarding the formulation and evaluation of Buccal drug delivery systems using sodium alginate and HPMC K 100M and serve as a reference for researchers who are involved in Buccal drug delivery research.

ACKNOWLEDGMENTS

The authors are grateful to Macleods Pharmaceutical Limited, Mumbai. for providing gift samples of Valsartan and P. Wadhwani college of Pharmacy for providing necessary facilities for the conduct of this work.

8. REFERENCES

1. Raghavendra Rao N.G, Shravani B, Reddy M.S. Overview on Buccal Drug Delivery Systems. J. Pharm. Sci. & Res., 2013; 5(4): 80–88.
2. Singh J, Deep P. A Review Article on Mucoadhesive Buccal Drug Delivery System.International Journal of P'ceutical Sciences and Research, 2013; 4(3): 916-927.
3. Bhowmik D, Sampath Kumar K.P, , Dutta A, Paswan S, Deb L. Recevt Advances In Mucoadhesive Buccal Drug Delivery Sysstem and its Matketed Scope And Opportunities.

Critical Review In Pharmaceutical Sciences, 2012; 1(1): 79-93.

4. Sutar P.S, D' Souza A, Patel S, Sambrekar A. The Design of Buccal Bioadhesive DrugDelivery System: A Review. Universal Journal of Pharmacy, 2013; 2(3): 25-37.
5. Baviskar D, Khairnar A, Jain D. Development of mucoadhesive buccal patch containing Aceclofenac:in vitro evaluation. International Journal of P'ceutical Sciences, 2009; 1(1): 91-95.
6. Deshmane S.V., channawar M.A, chandewar A.V, Joshi U.M, Biyani K.R. Chitosan based sustained release mucoadhesive buccal patches containing Verapamil HCL.
7. International Journal of Pharmacy and P'ceutical Sciences, 2009; 1(1): 216-229
8. Koland M, Charyulu R.N, Prabhu P. Mucoadhesive films of Losartan Potassium for Buccal delivery: Design and Characterization. Indian J.Pharm. Educ. Res., 2010; 44(4): 315-123.
9. Parikh B. A. Design and evaluation of buccal patches of valsartan. IJPI's Journal of
10. Pharmaceutics and Cosmetology, 2011; 1(2): 51-55.
11. Bansal S, Bansal M, Garg G, Preparation and evaluation of buccoadhesive patches of an antihypertensive drug. American Journal of Phytomedicine and Clinical Therapeutics, 2013; 1(2): 240-255.
12. Velmurugan S, Srinivas P. Formulation and on vitro evaluation of Losartan Potassium mucoadhesive buccal tablet. Asian J Pharm Clin Res., 2013; 6(3): 125-130.