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Design, Physicochemical Characterization and Pharmacokinetic Evaluation of Bilayered Buccal Tablets containing Statin Derivative

Shah Viral H[1*], Shelat Pragna[2], Shah Gaurang B[2]

[1] Dept. of Pharmaceutics, Sigma Institute of Pharmacy, Baroda, India
[2] Dept., Of Pharmaceutics, K.B.Institute of Pharmaceutical Education and Research, Gandhinagar, India

ABSTRACT

The objective of this study was to develop an effective Fluvastatin buccal adhesive tablet with excellent bioadhesive force and good drug stability in human saliva. The study also focuses on the mucoadhesive potential of some natural gums like tamrind gum, xanthan gum and gellan gum. Bilayered Buccoadhesive compacts with one layer of drug and mucoadhesive polymer and second non medicated, non permeable layer of ethylcellulose and Mg stearate was prepared using direct compression technique. Physicochemical properties of tablets like bioadhesive strength, swelling rate, surface pH, permeation rate and in vitro drug release rate were studied. As bioadhesive additives for tablets, a mixture of polymers like some natural gums along with chitosan was selected. Release studies revealed that the sustained release of Fluvastatin over several hours may be obtained by combining the chitosan with natural gums. Also the bioavailability studies indicated that bioavailability of Fluvastatin was enhanced using the above mentioned drug delivery system. Thus the potential of above mentioned drug delivery device is promising and may be considered as a novel tool in order to improve the therapeutic efficacy of various drugs with shorter half life and poor bioavailability.
Keywords: Mucoadhesion, Buccal drug delivery, Bilayered compact, Fluvastatin, Natural gums.

*Corresponding author
Email: ViralShah779@yahoo.com

INTRODUCTION

The oral cavity is being increasingly used for the administration of the drugs, which are mainly designed for the delivery of contained medicaments through the oral mucosa into the systemic circulation. Buccal mucosa consisting of stratified squamous epithelium supported by a connective tissue lamina propia was investigated as a site for drug delivery several decades ago and the interest in this area for the transmucosal drug administration is still growing. 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. Delivery of drugs through buccal mucosa overcomes premature drug degradation due to harsh environmental conditions within the GI tract, as well as active drug loss due to first pass metabolism and inconvenience of parenteral administration is also avoided. In addition there is excellent acceptability and drug can be applied, localized and may be removed easily at any time during the treatment period. However the conventional buccal dosage forms show limitations due to involuntary swallowing of the dosage form itself or a part of it may get dissolved and diluted by the salivary flow and will not be available for transmucosal absorption [1].

From technological point of view an ideal buccal dosage form must have three properties: i) it must maintain its position in mouth for a few hours. ii) It should release the drug in a controlled manner. I iii) It should provide the release in a unidirectional way towards the mucosa. [2] All the above mentioned properties can be achieved by developing a buccal mucoadhesive system with a non permeable backing layer. Bioadhesion, in particular mucoadhesion, has been area of interest for the development of controlled drug delivery systems to improve buccal, nasal and oral administration of drugs. Mucoadhesion can be explained by two major phenomenons. [3] The first is the formation of electrostatic, hydrophobic or hydrogen bonds at the interface between the polymer and mucin. The second is the diffusion of polymer chains in the mucus layer. [4]

Hyperlipidemia is a major cause of atherosclerosis and its associated disorders like coronary heart diseases, ischemic cerebrovascular diseases etc. Recognition of hypercholestermia as a risk factor has led to the development of drugs that reduces cholesterol levels. Statins are the most effective antihyperlipidemic agents. Statins act as competitive inhibitors of HMG-CoA reductase which catalyzes the step of cholesterol synthesis. Statins also reduces the triglycerides levels caused by elevated VLDL levels. All the statins are subjected to extensive first past metabolism by liver and gut wall enzymes, resulting in low systemic availability of the parent compound. Fluvastatin is also administered in its active form as a sodium salt and is almost completely absorbed, but 50-80% of the absorbed drug undergoes first pass metabolism whereby it is converted to its inactive metabolites which have a very short elimination half life.

The main objective of the study was to enhance the bioavailability of Fluvastatin by developing a bilayered buccal mucoadhesive compact of the drug using different natural mucoadhesive polymers.

MATERIALS ANS METHODS

MATERIALS

Chitosan (molecular mass: 400 kDa, 85% deacylated) was obtained from Central Fisheries Cochin, India. Xanthan Gum, Gellan Gum and Tamrind Gum were purchased from Loba Chem. Mumbai, India. All the other compounds, reagents polymers and solvents were purchased from Sigma Aldrich, Mumbai, India.

METHODS

Standardization of Gums

Standardization of natural gums was done based on following evaluation parameters like loss on drying, total ash value, viscosity of 1 % solution, particle size, pH of 1 % solution and microbial load.

In vitro mucoadhesive strength determination of polymers. [5, 6]

Rotating cylinder method

In this method 50 mg of the polymer was compressed in to 5.0 mm diameter disc, then these discs so prepared were adhered to the freshly excised gastric mucosa of male Albino rats by just hydrating the discs with little amount of water and placing them on stomach mucosa by applying little pressure. The whole system was pasted on the stainless steel cylinder of USP XXVI apparatus (type 4) with the aid of the cyanoacrylate glue and the cylinder was immersed in the dissolution jar filled with phosphate buffer pH 7.2 at 37ºC and was agitated at 125 rpm as shown in Fig.1 and the time for the detachment, disintegration or erosion of the test discs was monitored and reported in table 1..

Table 1: In Vitro Mucoadhesive strength of Polymers

POLYMER	DISK DETACHMENT TIME (HOURS) IN PH 7.2 BUFFER $\pm$ S.D.
Chitosan	2.35 $\pm$ 0.52 (disk disintegrates)
Xanthan Gum	18.25 $\pm$ 0.42
Tamrind Gum	15.15 $\pm$ 0.25
Gellan Gum	18.30 $\pm$ 0.50

Interaction Studies

Drug Polymer Interaction studies were performed using FTIR spectroscopy.
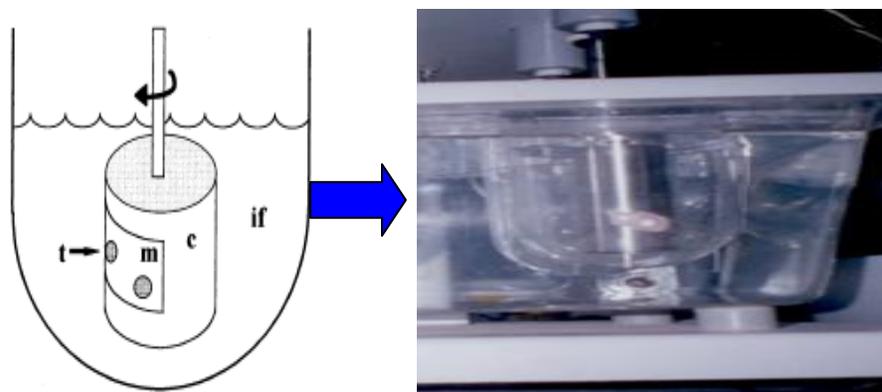
Figure 1: Schematic presentation of the test system used to evaluate the mucoadhesive properties of tablets based on various polymers. c, cylinder; if, intestinal fluid; m, rat mucosa; t, tablet.

Preparation of   bilayered mucoadhesive buccal compacts. [7, 8, 9, 10, 11]

Bilayered compacts were prepared by a direct compression procedure involving two consecutive steps. The non medicated layer was first compressed then the medicated layer was filled into the die cavity and both layers were compressed together. In the first step the backing membrane was created by blending the ethyl cellulose and Mg stearate mixture and the blended powder of backing layer was then compressed using flat faced punch, 9 mm in diameter. In the second step the mucoadhesive polymer/drug mixture was prepared by homogeneous mixing in mortar pestle for 15 mins. The mixture was then filled in the die cavity and was compressed on previously obtained backing layer. Various formulations consisting of different polymers in varied composition were prepared as shown in the table 2.

Table 2: Composition of bilayered mucoadhesive buccal compacts.

Components (mg)	FORMULATIONS												
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
Fluvastatin	40	40	40	40	40	40	40	40	40	40	40	40	40
Backing layer													
Magnesium Stearate	25	25	25	25	25	25	25	25	25	25	25	25	25
Ethyl Cellulose	25	25	25	25	25	25	25	25	25	25	25	25	25
Aluminium Hydroxide (stabilizer)	5	5	5	5	5	5	5	5	5	5	5	5	5
Chitosan	50	100	---	---	---	---	---	---	---	---	---	100	100
Tamrind Gum	---	---	50	100	125	---	---	---	---	---	---	125	---
Xanthan Gum	---	---	---	---	---	50	100	125	---	---	---	---	125
Gellan Gum	---	---	---	---	---	---	---	---	50	100	125	---	---

Evaluation of bilayered mucoadhesive buccal compacts. [12, 13]

Bilayered compacts so prepared were evaluated for following preliminary evaluation tests like hardness, weight variation, friability, thickness, mucoadhesive strength, permeation studies and In vitro release studies. Three individual compacts from each batch were used and the results were averaged. Hardness was determined using a Monsanto type of hardness tester. Weight variation was determined as per USP were the tolerance limit was 7.5%. Friability was determined using a Roche type of friabilator. Thickness of buccal compact was determined using digital vernier caliper as indicated in table 3.

Table 3: Evaluation test results of bilayered mucoadhesive buccal compacts.

Evaluation Tests	Test Results of various Formulations												
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
Hardness (Kg/cm2)	4.4	4.6	4.4	4.2	4.8	4.5	4.6	4.8	4.4	4.6	4.6	4.4	4.4
Friability (%)	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Weight variation	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Thickness (mm)	1.2	1.5	1.3	1.6	1.8	1.4	1.6	1.4	1.6	1.8	1.8	2.0	2.0

In vitro release studies [14]

The in vitro drug release studies of buccal compacts were carried out using USP dissolution apparatus 1. In order to mimic the in vivo adhesion of the devices, the buccal compact was attached through cyanoacrylate glue to the bottom end of the stirring rod instead of basket fixtures. By this only peripheral layer of the buccal compact was exposed to the dissolution medium. The rotation speed was kept to be 50 rpm and 500 ml phosphate buffer pH 6.6 was used as the dissolution medium maintained at $37\pm0.5°C$. Aliquots were withdrawn at different time intervals and analyzed spectrophotometrically at 238nm. The dissolution studies were conducted in triplicates and the mean values were plotted verses time with standard error, indicating the reproducibility of results as indicated in fig. 2.

In vitro Drug Permeation Studies [15, 16, 17]

From the local slaughter house porcine buccal mucosa was collected and was immediately transported to the laboratory in cold normal saline solution. The buccal mucosa, with a part of sub mucosa was carefully separated from fat and muscles using scalpel. The buccal epithelium was used within two hours after removal. The in vitro buccal drug permeation study was performed using a Franz diffusion cell at 37°C ± 0.2°C. Buccal mucosa was mounted between the donor and receptor compartments. The receptor compartment (20-

ml capacity) was filled with phosphate buffer pH 6.6. The buccal mucosa was allowed to stabilize for a period of one hour. The buccal compact was placed with the core facing the mucosa, and the compartments were clamped together. The hydrodynamics in the compartment was maintained by stirring with a magnetic bead at uniform slow speed. Samples were withdrawn at predetermined time intervals and analyzed for drug content by UVspectrophotometer.
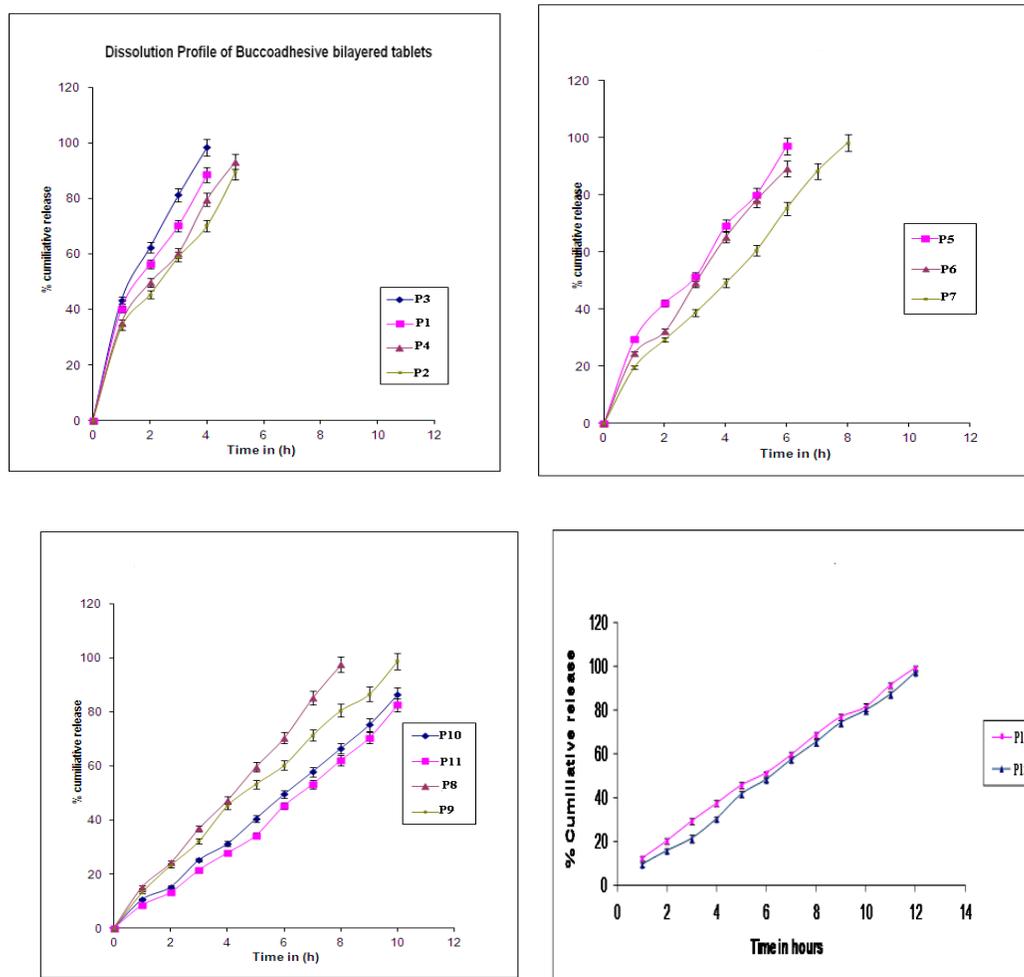

Figure 2: Dissolution profiles of bilayered mucoadhesive buccal compacts.

Swelling Study [18, 19]

Buccal compacts were weighed individually (W1) and placed separately in 2% agar gel plates with the core facing the gel surface and incubated at 37°C ± 1°C. After 6 hours, the compact was removed from the Petri dish and excess surface water was removed carefully using filter paper. The swollen compact was then reweighed (W2), and the swelling index (SI) was calculated using the following formula and results were reported in table 4.

$$SI = \frac{(W2 - W1)}{W1} \times 100$$

Table 4: Swelling Index and surface pH determination of bilayered mucoadhesive buccal compacts

Formulation code	Swelling Index	Surface pH
P12	38 $\pm$ 0.50	6.5 $\pm$ 0.3
P13	35 $\pm$ 0. 20	6.7 $\pm$ 0.2

Surface pH Study [18, 19]

The surface pH of the buccal compacts was determined in order to investigate the possibility of any side effects in vivo. As an acidic or alkaline pH may irritate the buccal mucosa, so the surface pH should be as close to neutral as possible. The method adopted by Bottenberg et al. was used to determine the surface pH of the compact. A combined glass electrode was used for this purpose. The compact was allowed to swell by keeping it in contact with 1 mL of distilled water for 2 hours at room temperature. The pH was identified by bringing the electrode into contact with the compact surface and allowing the surface to equilibrate for 1 minute. The results were reported in table 4.

In vitro mucoadhesive strength determination [20, 21]

In vitro mucoadhesive strength of the optimized formulation was determined using time based and forced based technique and the results were reported in table 5.

Table 5: In vitro mucoadhesive strength determination of optimized formulations.

Formulation code	Detachment time (hours)	Detachment force(g/cm^2)
P12	12 (disk remained undetached)	92.7 $\pm$ 0.3
P13	12(disk remained undetached)	98.5 $\pm$ 0.5

Force based technique [22]

Tensile experiments were done on Instron app. (Model 4301), using porcine buccal mucosa. Cynoacrylate glue was used to fix the compact and the porcine mucosa to the upper and lower metalic supports respectively. 20µl of distilled water was dropped on the compact surface and the compact and the mucosa was brought in contact with a force of 0.5N and kept in this condition for 10 mins. Then the tensile experiment was performed at a constant extension rate of 5mm/min.

Time based Technique

It was performed using rotating cylinder method as mentioned above.

Bioavailability Assessment of Fluvastatin

The potential of the mucoadhesive buccal compacts to deliver Fluvastatin to the systemic circulation in a sustained fashion was evaluated by conducting the bioavailability experiments. Two groups of rabbits were taken, each group consisting of three rabbits in the weight range of 2.5-3kgs. Animals were anesthetized by an i.m injection of 1:5 mixture of Xylazine (1.9mg/kg) and ketamine (9.3 mg/kg). The rabbits were fasted for 12 h before until the end of the experiment. To one group of rabbits marketed Fluvastatin compacts LESCOL® were given and to the other group buccal adhesive compacts were placed on the upper gingiva. From both the groups 2-ml blood samples were collected before the administration of the compacts and at time intervals of 1, 2, 4, 6, 8, and 12h respectively. The catheter was placed in the marginal ear vein for blood collection when the rabbits were anesthetized. After the collection of the blood sample every time the cannula was flushed with 0.2ml of a 10% (v/v) heparin solution to keep the cannula open. All the blood samples were centrifuged at 3000 rpm for 10 mins to separate plasma. The retrived plasma was stored at -20°C until the time of analysis.

Quantitation of Plasma Fluvastatin

Fluvastatin was quantified using a Shimadzu high performance liquid chromatography (HPLC) system consisting of an ultraviolet detector. The Class LC10 software version 1.6(Shimadzu) was used for data analysis and processing. The compounds were separated at 50 °C on a C18 column (5m, 250×4.6mm I.D.) with guard column and quantified by ultraviolet detection at 304 nm. For preparation of the mobile phase, 200 ml of acetonitrile was mixed with 300 ml of 0.05M potassium dihydrogen phosphate buffer (pH 5) and 500 ml of methanol. The mobile phase was delivered at a flow rate of 1.2 ml/min. The substances were quantified using their peak area ratio to the internal standard. In a polypropylene tube, 1.0 ml plasma was mixed with 1ml of acetonitrile and was mixed for 5s to it 1 ml of internal standard (1µg Atrovastatin/1 ml methanol), 2 ml of phosphate buffer and 10ml of methanol was added. The proteins were precipitated under agitation for 20 min at room temperature. Samples were spun for 10 min at 3000 rpm, supernatants were transferred into a glass tube and evaporated to dryness at 40°C under a stream of nitrogen. Prior to HPLC estimation 0.4ml of mobile phase was added to the sample.

Preparation of stock solutions and calibration standards:

A stock solution was prepared by dissolving 40 mg Fluvastatin in 80 methanolic solution in a 50ml volumetric flask. The working solution was obtained by dilution with methanol to a final concentration of 80 µg/ml. This solution was used for preparation of calibration standards and quality control samples. For the preparation of the internal standard solution, 0.1 mg Atrovastatin was dissolved in methanol in a 100ml volumetric flask. For calibration standards, 100 µl working solution was evaporated to dryness and reconstituted in 20 ml blank human plasma yielding the highest calibration standard with a concentration of 1.2 µg/ml Fluvastatin, which was then used to generate standard samples with final Fluvastatin concentrations of 0.2,

0.4,0.6,0.8,1.0,1.2 μg/ml by serial dilution with blank plasma. Calibration standards, and blank plasma samples were stored at −20 °C until analysis.

Stability studies

Stability studies were carried out for optimized formulations as per ICH guidelines by placing the formulations at 40°C / 75% RH for 90 days in stability chamber. The formulation was then characterized for its various physical and pharmaceutical parameters after stability studies.

Estimation of Pharmaceutical Characteristics After Stability Studies as shown in Table 8 and 9 and fig, 8 and 9.

Table 6: Calibration Standard for Plasma Fluvastatin Estimation.

SLOPE	INTERCEPT	r^2
0.58	0.0023	0.9985

Table 7: Comparison of Pharmacokinetics of Fluvastatin after oral administration and buccal administration

PHARMACOKINETIC PARAMETER	ORAL ADMINISTRATION OF FLUVASTATIN	BUCCAL ADMINSTRATION OF FLUVASTATIN
C_{max} (ng/ml)	76	73
t_{max} (h)	1.0	4.0
AUC_{0-12} (ng/ml per h)	225	493

Table 8: Estimation of physical and chemical characteristics after stability studies

Formulations	Physical parameters			Chemical parameters		
	Colour			Drug content		
Sampling Points	0th Month	3rd Month	6th Month	0th Month	3rd Month	6th Month
Bulk drug	No change	No change	No Change	_	_	_
P12	No change	No change	No change	90.56 + 0.5%	91.23+1%	97.57+0.5%
P13	No change	No change	No change	89.14 + 0.7%	93.16 + 0.5%	90.14 + 0.7%

Table 9: Evaluation of pharmaceutical parameters after stability studies

Formulation code	AVERAGE ADHESION FORCE (g/cm^2)	Swelling Index	Surface pH
P12	95.6+ 0.3	35 + 0.61	6.4 + 0.2
P13	99.3 + 0.17	30.8 + 0.52	6.5 + 0.4

RESULTS AND DISCUSSIONS

In vitro mucoadhesive strength determination of polymers (Rotating cylinder method) t

The in vitro mucoadhesive test results as mentioned in Table1. showed that Chitosan disc erroded quickly allthough it was not detached from the mucosa. Natural gums like xanthan gum, gellan gum and tamrind gum showed good mucoadhesive strength.

Evaluation of bilayered mucoadhesive buccal compacts.

Interaction studies

FTIR graphs revealed that there was no interaction between drug and polymer.

In vitro drug release studies and In vitro drug permeation rate studies.

The in vitro drug release and drug permeation studies results as indicated in fig. 2 and fig. 3 showed that use of plain chitosan as mucoadhesive polymer could not sustain the release of the drugs from the formulation.. Xanthan gum and tamrind gum showed sufficient sustaining properties but consecutive low permeation properties so the combination of chitosan and xanthan gum as well as tamrind gum resulted into a formulation which sustains the release of the drug from the formulation and also have high permeation rate.
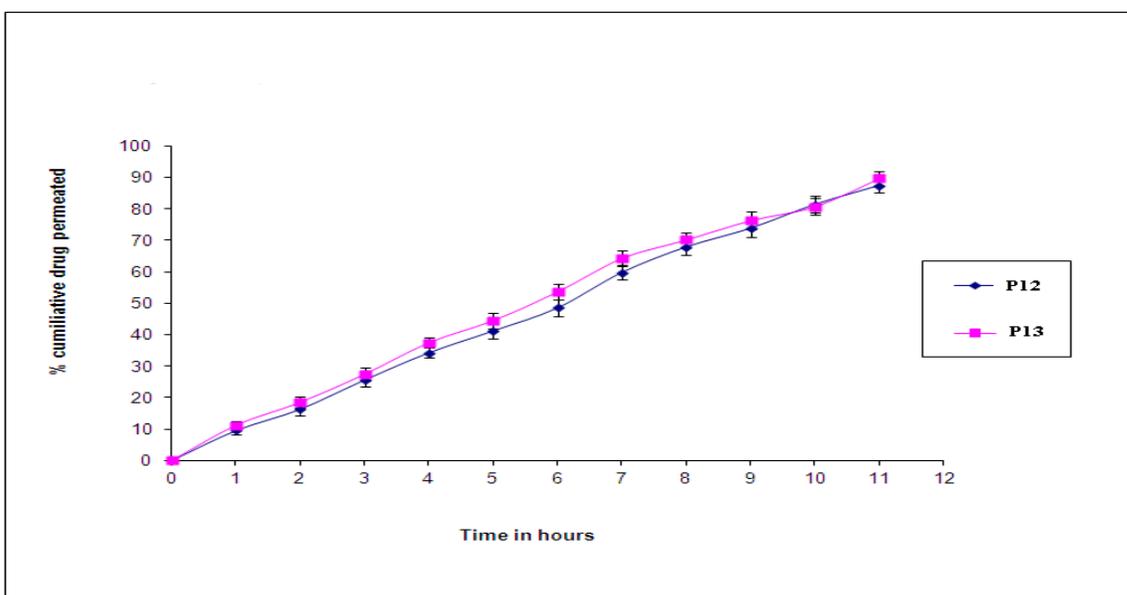

Figure 3: In vitro drug permeation profile of bilayered mucoadhesive buccal compacts

Swelling properties, Surface pH and in vitro mucoadhesive strength determination studies.

The swelling study results as indicated in table 4. Indicated that the optimized formulation has sufficient swelling character which is essential for good mucoadhesive properties as more will be the swelling , greater will be the exposure of the formulation to the biological surface and more will be the mucoadhesion. The surface pH of the optimized

formulations was found to be in the range of buccal pH which indicated that there will be no irritation due to formulation on the buccal surface. Also the forced based and time based mucoadhesive strength determination studies as shown in Table 5. Indicated that optimized formulation showed good mucoadhesive strength.

Bioavailability Assessment of Fluvastatin

The Mean plasma conc. – time curve of Fluvastatin from marketed formulation (LESCOL ®) and from buccal mucoadhesive bilayered compacts as shown in fig. 5 indicated that AUC of the mucoadhesive formulation was higher which indicated in enhanced bioavailability of Fluvastatin in mucoadhesive buccal compacts when compared to conventional Fluvastatin tablets.

Stability studies

The physical, chemical and pharmaceutical evaluation studies of the optimized formulation after stress conditions as indicated in table 8 and table 9 and fig.6 and fig. 7 revealed that the optimized formulation is sufficiently stable.
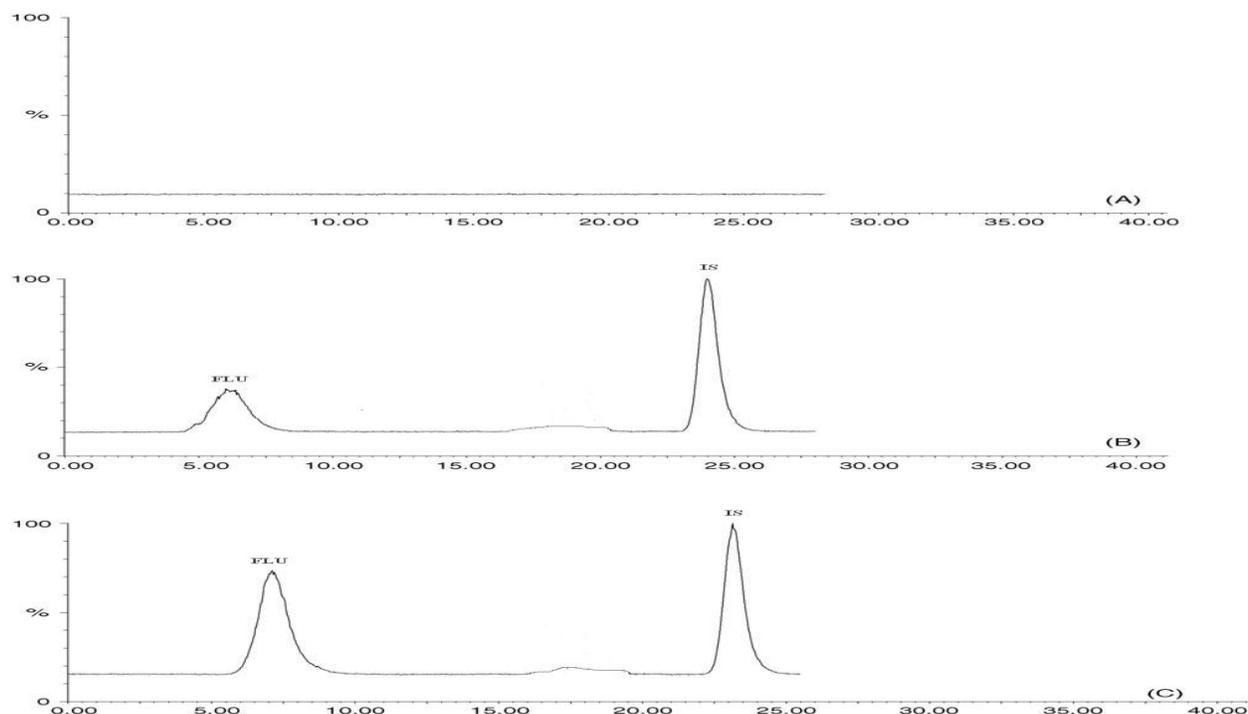

Figure 4: HPLC trace of Fluvastatin (FLU) and the internal standard Atrovastatin (IS) using ultraviolet detection at 304nm .(A) Blank Plasma sample. (B) Plasma sample post 2 hours of administration of 40 mg LESCOL ® c)Plasma sample post 2 hours of administration of 40 mg Fluvastatin adhesive tablets.
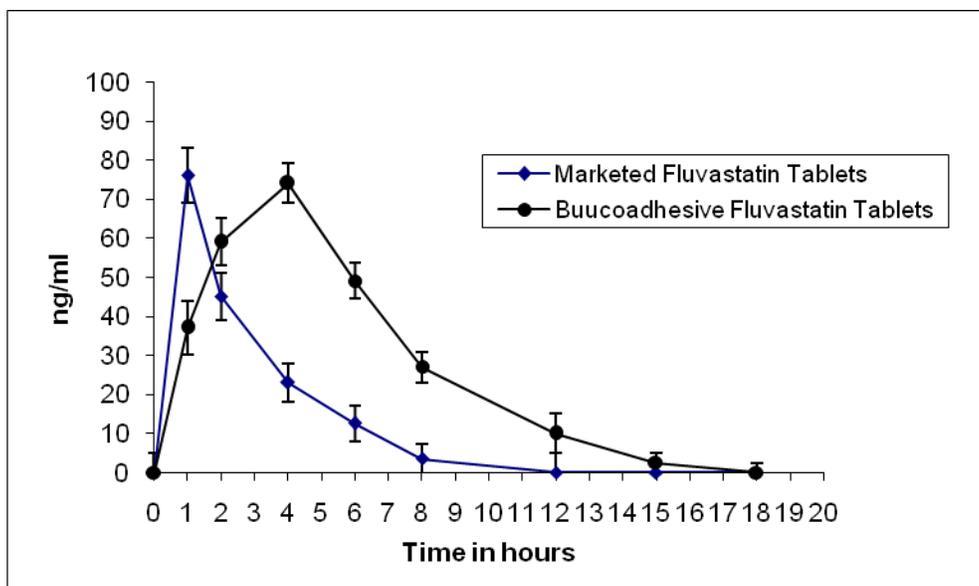
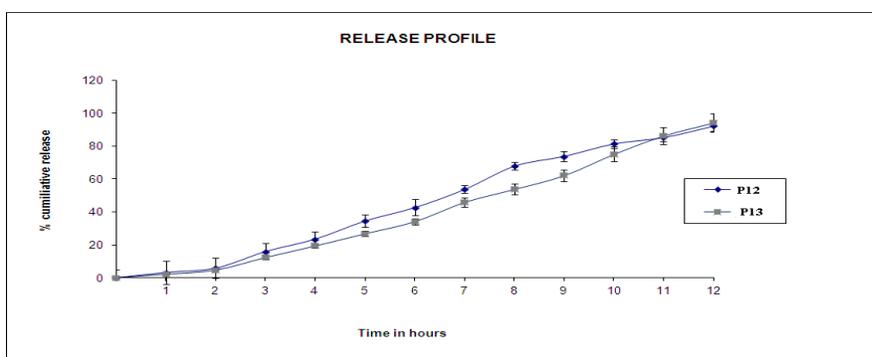**Figure 5: Mean plasma conc. –time curve of Fluvastatin**

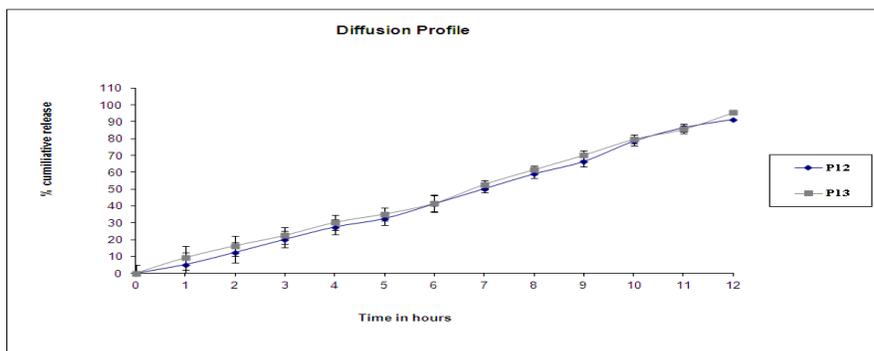**Figure 6: In vitro release after stability studies**

Figure 7: In vitro Drug Permeation after stability studies

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