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Full Length Research Paper

Design and evaluation of thiolated chitosan based mucoadhesive and permeation enhancing bilayered buccal drug delivery system

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The objective of this study was to increase the bioavailability of fluvastatin by developing bilayered buccal mucoadhesive compacts. This study also focuses on the mucoadhesive potential of some natural gums for improved mucoadhesion and transmucosal permeation of existing mucoadhesive polymers by certain modifications. Bilayered mucoadhesive compacts with one layer of drug and mucoadhesive polymer, and second, non medicated, non permeable layer of ethylcellulose and magnesium stearate was prepared using direct compression technique. Natural gums like tamrind and xanthan gum were evaluated for its mucoadhesive properties. The mucoadhesion along with permeation character of chitosan was enhanced by immobilization of thiol groups on its surface utilizing 2-iminothiolane (Trauts reagent). The resulting chitosan-4thio-butylamidine conjugate (chitosan 4-thiobutylamidine (TBA) conjugate) was evaluated for its mucoadhesion, permeation and release properties. Experimental data revealed a several fold higher mucoadhesive property of chitosan-TBA conjugate than unmodified chitosan along with good permeation properties. Release studies revealed that the sustained release of fluvastatin over several hours may be obtained by combining the chitosan TBA conjugate with natural gums like xanthan and tamrind gum. Also, the bioavailability studies indicated that bioavailability of fluvastatin was enhanced using the aforementioned drug delivery system. Thus, the potential of the aforementioned 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.

Key words: Mucoadhesion, buccal drug delivery, bilayered compact, thiolated chitosan.

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 gastrointestinal (GI) tract, as well as active drug loss due to the 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

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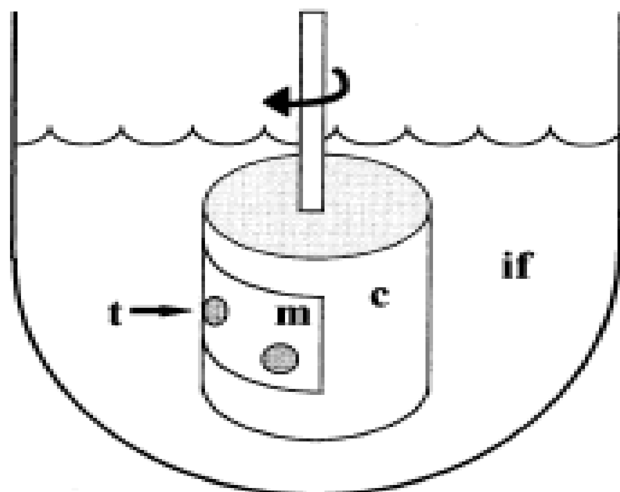

Figure 1. Schematic presentation of the test system used to evaluate the mucoadhesive properties of tablets based on various polymers. c, Cylinder; if, intestinal fluid; m, rat mucosa; t, tablet.

salivary flow and will not be available for transmucosal absorption (Yajaman et al., 2006).

From technological point of view, an ideal buccal dosage form must have three properties: (1) It must maintain its position in mouth for a few hours; (2) It should release the drug in a controlled manner, and (3) It should provide the release in a unidirectional way towards the mucosa (Nazila et al., 2005). All the aforementioned 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 pheno-mena (Shimona et al., 2004). 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 (Patel et al., 2005).

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 very low density cholesterol (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 to 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 present 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

Chitosan (molecular mass: 400 kDa, 85% deacylated) was obtained from Central Fisheries Cochin, India. Trauts reagent was gifted by Merck, Switzerland. Xanthan gum and tamarind gum were purchased from Loba Chem. Mumbai, India. All the other compounds, reagents polymers and solvents were purchased from Sigma Aldrich, Mumbai, India.

Standardization of gums

Standardization of natural gums was done based on the 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

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 37°C and was agitated at 125 rpm as shown in Figure 1 and the time for the detachment, disintegration or erosion of the test discs was monitored and reported as shown in Table 1 (Llabot et al., 2007; Luana et al., 2004).

Interaction studies

Drug polymer interaction studies were performed using Fourier transform infrared (FTIR) spectroscopy.

Preparation of bilayered mucoadhesive buccal compacts

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 min. 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 Table 2 (Jafar et al.,

Table 1. *In vitro* mucoadhesive strength of polymers.

Polymer	Disk detachment time (h) in pH 7.2 buffer ±SD
Chitosan	2.35 ± 0.52 (disk disintegrates)
Xanthan gum	18.25 ± 0.42
Tamarind gum	15.15 ± 0.25
Thiolated chitosan	28.30 ± 0.50

Table 2. Composition of bilayered mucoadhesive buccal compacts.

Component (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	-	-	-	-	-	-	-	-	-	-	-
Thiolated chitosan	-	-	50	100	125	-	-	-	-	125	125	125	125
Xanthan gum	-	-	-	-	-	50	100	-	-	125	-	150	-
Tamarind gum	-	-	-	-	-	-	-	50	100	-	125	-	150

2004; Musnasur et al., 2006; Narendra et al., 2005, 2006; Noha et al., 2004).

Modification of chitosan with 2-iminothiolane HCl

Five gram of chitosan (degree of deacetylation: 83 to 85%) was dissolved in 700 ml of 1% acetic acid for 5 h, and to it 2 g of 2-iminothiolane HCl (Traut's reagent) was added, and then after an incubation period of 24 h at room temperature, under continuous stirring the resulting polymer conjugates were dialyzed against 5 mM HCl, two times against 5 mM HCl containing 1% NaCl, against 5 mM HCl and finally against 1 mM HCl. Thereafter, samples were lyophilized by drying frozen aqueous polymer solutions at -40°C and 0.01 mbar, and were stored at 4°C until further use. As indicated in Figures 2 and 3, the additional peaks in the FTIR Spectra of modified chitosan in the region of 600 to 800 revealed the presence of thiol group (Andreas et al., 2004; Nina et al., 2009).

Evaluation of bilayered mucoadhesive buccal compacts

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 where 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 (Chul et al., 2001).

In vitro release studies

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 ± 0.5°C. Aliquots were withdrawn at different time intervals and analyzed spectrophotometrically at 238 nm. The dissolution studies were conducted in triplicates and the mean values were plotted versus time with standard error indicating the reproducibility of results as Indicated in Figure 4.

In vitro drug permeation studies

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 2 h after removal. The *in vitro* buccal drug permeation study was performed using a Franz diffusion cell at 37 ± 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 1 h. 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 UV spectrophotometer (Verma and Chattopadhyay, 2011; Giuseppina et al., 2004; Mia and Tuononen, 2003).

Swelling study

Buccal compacts were weighed individually (W1) and placed

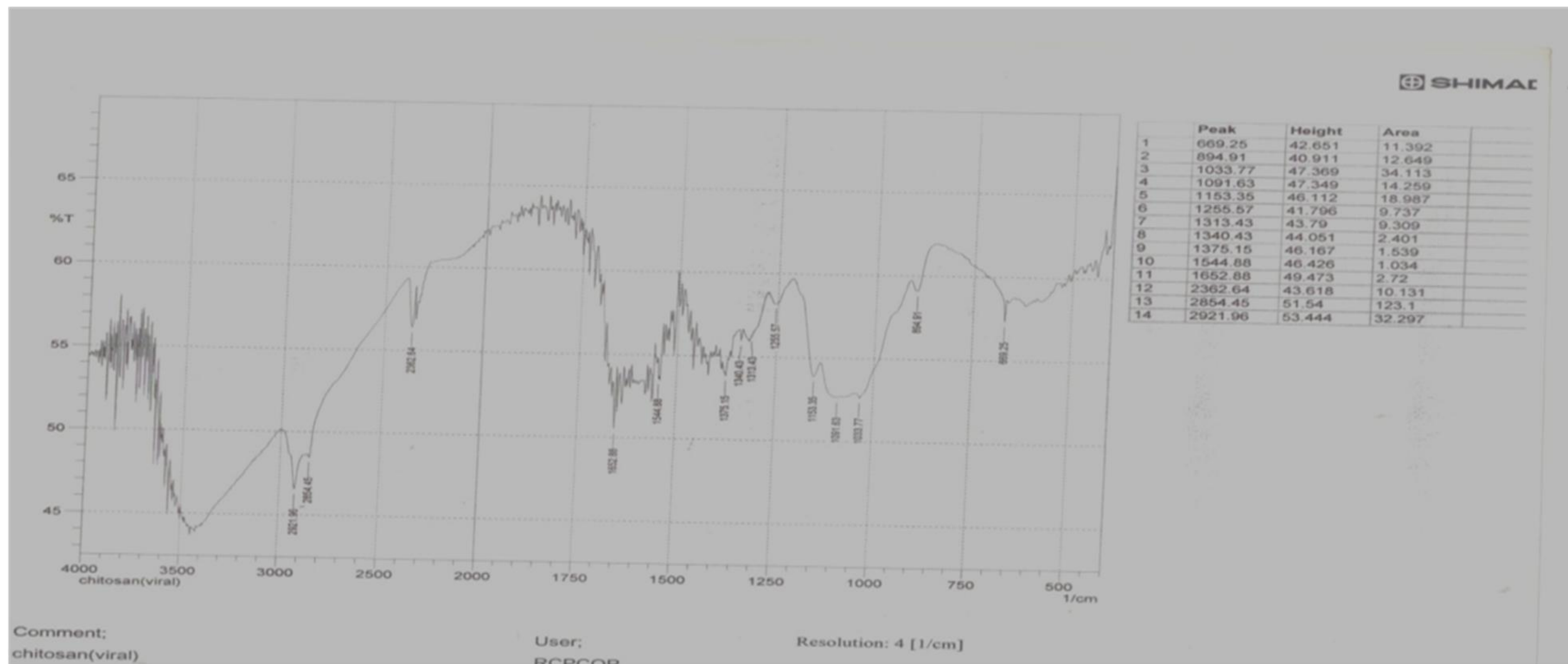

Figure 2. Spectra of chitosan.

separately in 2% agar gel plates with the core facing the gel surface and incubated at 37 ± 1°C. After 6 h, 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 as shown in Table 4 (Paolo et al., 2008; Soliman et al., 2005).

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

Surface pH study

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., (1991) 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 h at room temperature. The pH was dentified by bringing the electrode into contact with the compact surface and allowing the surface to equilibrate for 1 min. The results have been reported as shown in Table 4 (Paolo et al., 2008; Soliman et al., 2005).

***In vitro* mucoadhesive strength determination**

In vitro mucoadhesive strength of the optimized formulation was determined using time based and forced based technique and the results have been reported as shown in Table 5 (El-Samaligy et al., 2004; Ramesha et al., 1999).

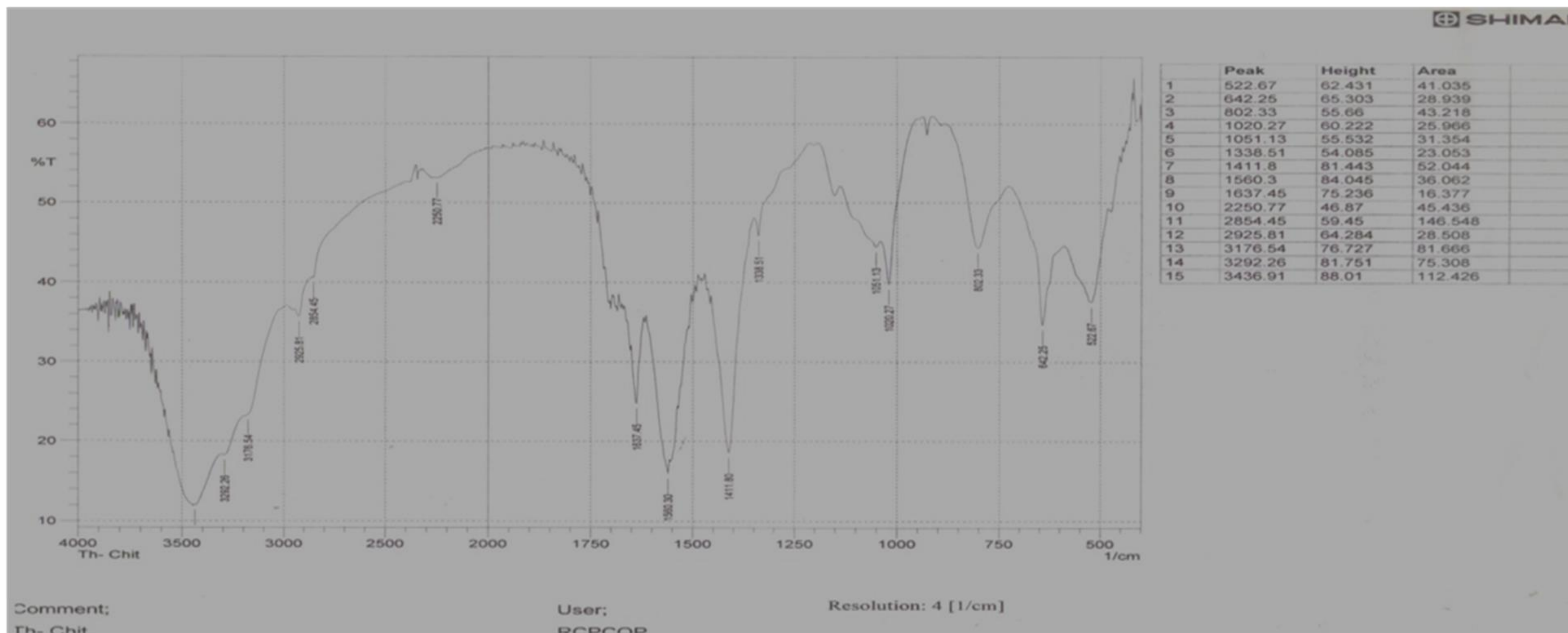

Figure 3. Spectra of thiolated chitosan.

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

Evaluation test	Test results of various formulations												
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13
Hardness (kg/cm^2)	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

Force based technique

Tensile experiments were done on Instron app. (Model 4301), using porcine buccal mucosa. Cyanoacrylate glue was used to fix the compact and the porcine mucosa to the upper and lower metalic supports, respectively. 20 µl of distilled water was dropped on the compact surface, and the compact and the mucosa was brought in contact with a force of0.5 N and were kept in this condition for

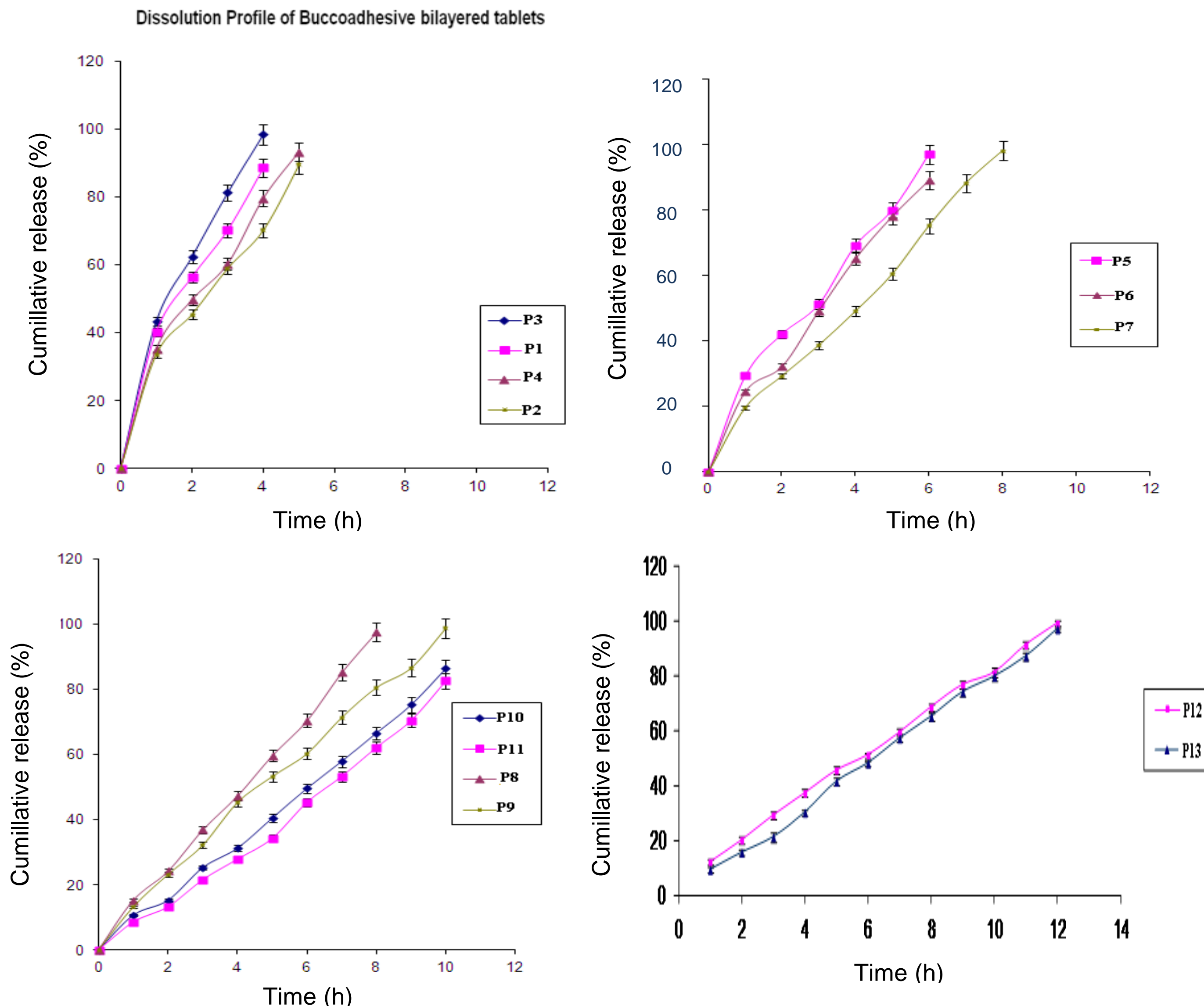

Figure 4. Dissolution profiles of bilayered mucoadhesive buccal compacts.

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

Formulation code	Swelling index	Surface pH
P12	38 ± 0.50	6.5 ± 0.3
P13	35 ± 0. 20	6.7 ± 0.2

10 min. Then, the tensile experiment was performed at a constant extension rate of 5 mm/min (Isabel et al., 2007).

Time based technique

This was performed using the aforementioned rotating cylinder method.

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 to 3 kg. Animals were anesthetized by an intramuscular (IM) injection of 1:5 mixture of xylazine (1.9 mg/kg) and ketamine (9.3 mg/kg). The rabbits were fasted for 12 h before and 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 groups, 2 ml blood samples were collected before the administration of the compacts and at time intervals of 1, 2, 4, 6, 8 and 12 h, respectively. The catheter was placed in the marginal ear vein for blood collection

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

Formulation code	Detachment time (h)	Detachment force (g/cm^2)
P12	12 (disk remained undetached)	92.7 ± 0.3
P13	12 (disk remained undetached)	98.5 ± 0.5

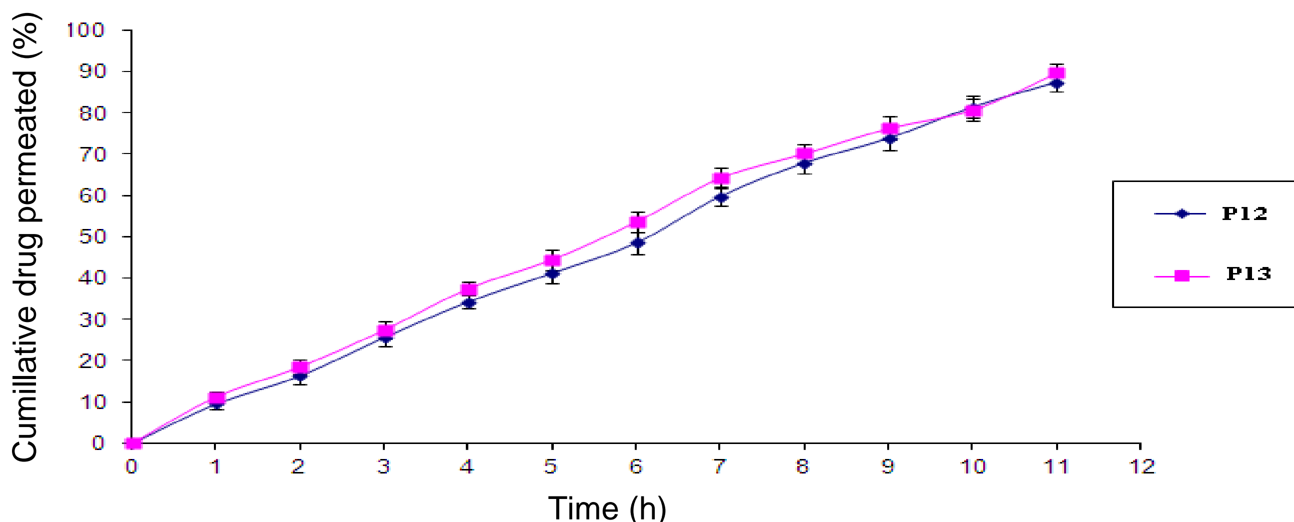

Figure 5. *In vitro* drug permeation profile of bilayered mucoadhesive buccal compacts.

when the rabbits were anesthetized. After the collection of the blood sample, every time the cannula is flushed with 0.2 ml of a 10% (v/v) heparin solution to keep the cannula open. All the blood samples were centrifuged at 3000 rpm for 10 min to separate plasma. The retrieved plasma was stored at -20°C until the time of analysis. The animal studies were approved by institutional ethics committee (Registration no. 934/A/06/CPCSEA). All the observed pahramcokinetic parameters of fluvastatin after oral and buccal administration are mentioned in Table 7.

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 C_{18} column (5 m, 250 × 4.6 mm I.D.) with guard column and were quantified by ultraviolet detection at 304 nm. For preparation of the mobile phase, 200 ml of acetonitrile was mixed with 300 ml of 0.05 M 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 1 ml of acetonitrile and was mixed for 5 s to it 1 ml of internal standard (1 µg atrovastatin/1 ml methanol), 2 ml of phosphate buffer and 10 ml 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, 0.4 ml of mobile phase was added to the sample (Figure 6).

Preparation of stock solutions and calibration standards

A stock solution was prepared by dissolving 40 mg fluvastatin in 80 methanolic solution in a 50 ml volumetric flask.

The working solution was obtained by dilution with methanol to a final concentration of 80 µg/ml. This solution was used for the preparation of calibration standards and quality control samples. For the preparation of the internal standard solution, 0.1 mg atrovastatin (Al-Rawithi et al., 2003) was dissolved in methanol in a 100 ml 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 and 1.2 µg/ml by serial dilution with blank.

RESULTS AND DISCUSSION

In vitro mucoadhesive strength determination of polymers (rotating cylinder method)

The *in vitro* mucoadhesive test results shown in Table 1 showed that chitosan showed the minimum mucoadhe-sion which was several folds enhanced by modyfing the chitosan by immobilizing thiol groups on its surface through Trauts reagent. Xanthan and tamarind gum showed sufficient muoadhsion power required to retain

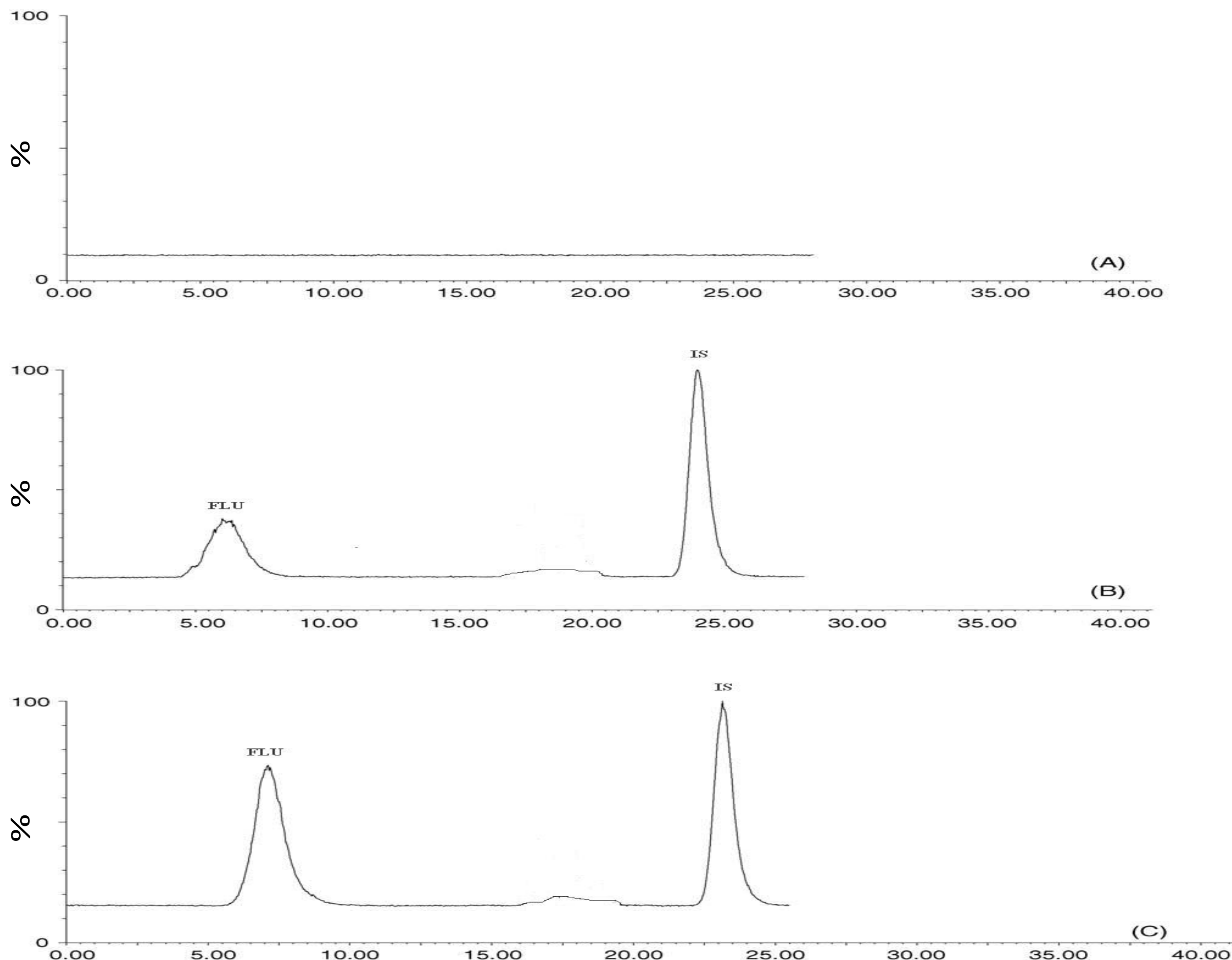

Figure 6. HPLC trace of fluvastatin (FLU) and the internal standard atrovastatin (IS) using ultraviolet detection at 304 nm. (A) Blank plasma sample. (B) Plasma sample post 2 h of administration of 40 mg LESCOL ®. (C) Plasma sample post 2 h of administration of 40 mg fluvastatin adhesive tablets.

the drug on the mucosal surface upto 12 h as mentioned in Table 1.

Evaluation of bilayered mucoadhesive buccal compacts

Interaction studies

FTIR graphs revealed that there was no interaction between drug and polymer as major drug peaks which were observed in the FTIR spectra of drug alone at wave numbers 3420, 1654, 1566, 1405, 1215, 1157, 840, 741 and 560 were also seen in the FTIR spectra of formulation containing drug along with mucoadhesive polymers and other excipients.

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 Figures 4 and 5 showed that use of plain chitosan as mucoadhesive polymer could not sustain the release of the drugs from the formulation. Immobilization of thiol groups on the surface of the chitosan further decreases the sustaining properties of the chitosan due to increase in hydrophillicity of the polymer. Xanthan and tamarind gum showed sufficient

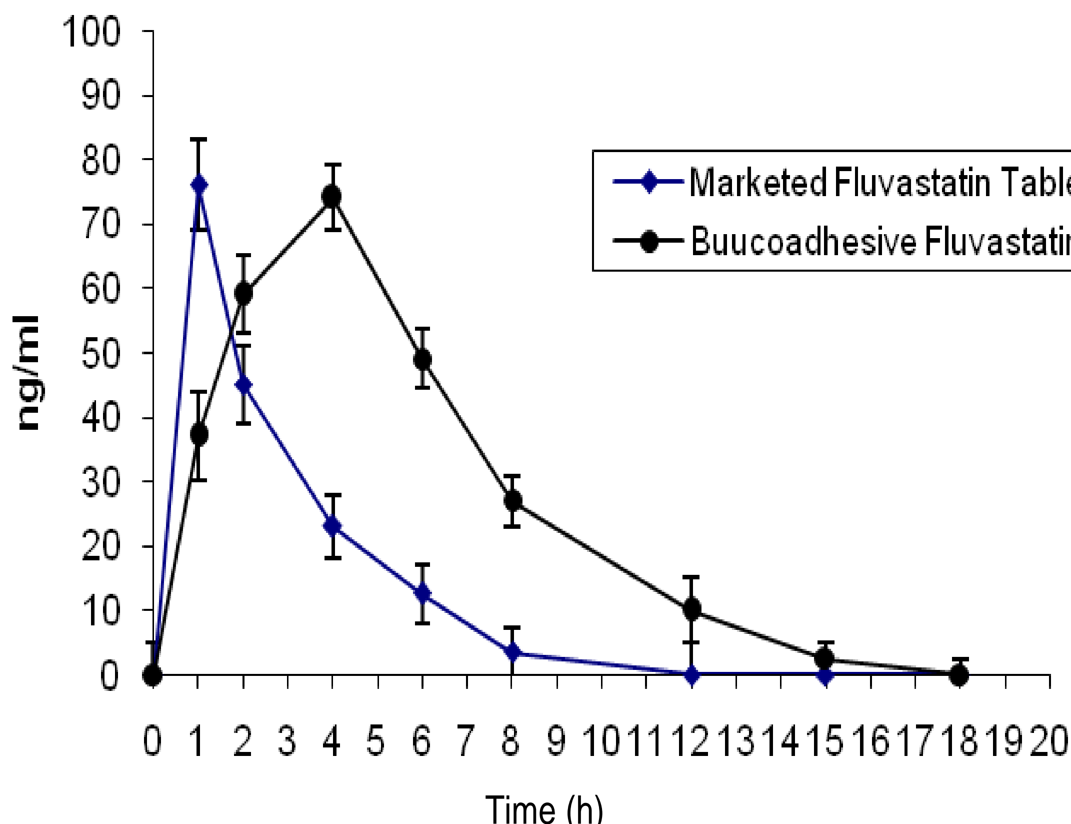

Figure 7. Mean plasma concentration-time curve of fluvastatin.

Table 6. Calibration curve data for plasma fluvastatin estimation.

Slope	intercept	R^2
0.58	0.0023	0.9985

sustaining properties but consecutive low permeation properties, so the combination of thiolated chitosan and xanthan gum as well as tamarind gum resulted in a formulation which sustains the release of the drug from the formulation and also have high permeation rate.

Swelling properties, surface pH and *in vitro* mucoadhesive strength determination studies

The swelling study results is as indicated in Table 4, which indicates 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 6 indicated that optimized formulation showed good mucoadhesive strength.

Bioavailability assessment of fluvastatin

The mean plasma concentration-time curve of fluvastatin from marketed formulation (LESCOL ®) and from buccal mucoadhesive bilayered compacts as shown in Figure 7 indicated that AUC of the muco-adhesive formulation was higher which indicated an 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 con-ditions as indicated in Tables 8 and 9 and Figures 8 and 9 revealed that the optimized formulation is sufficiently stable.

Table 7. Comparison of pharmacokinetics of fluvastatin after oral administration and buccal administration.

Pharmacokinetic parameter	Oral administration of fluvastatin	Buccal administration 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.

Formulation	Physical parameter			Chemical parameter		
	Colour			Drug content ± SD (three observations)		
Sampling point	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

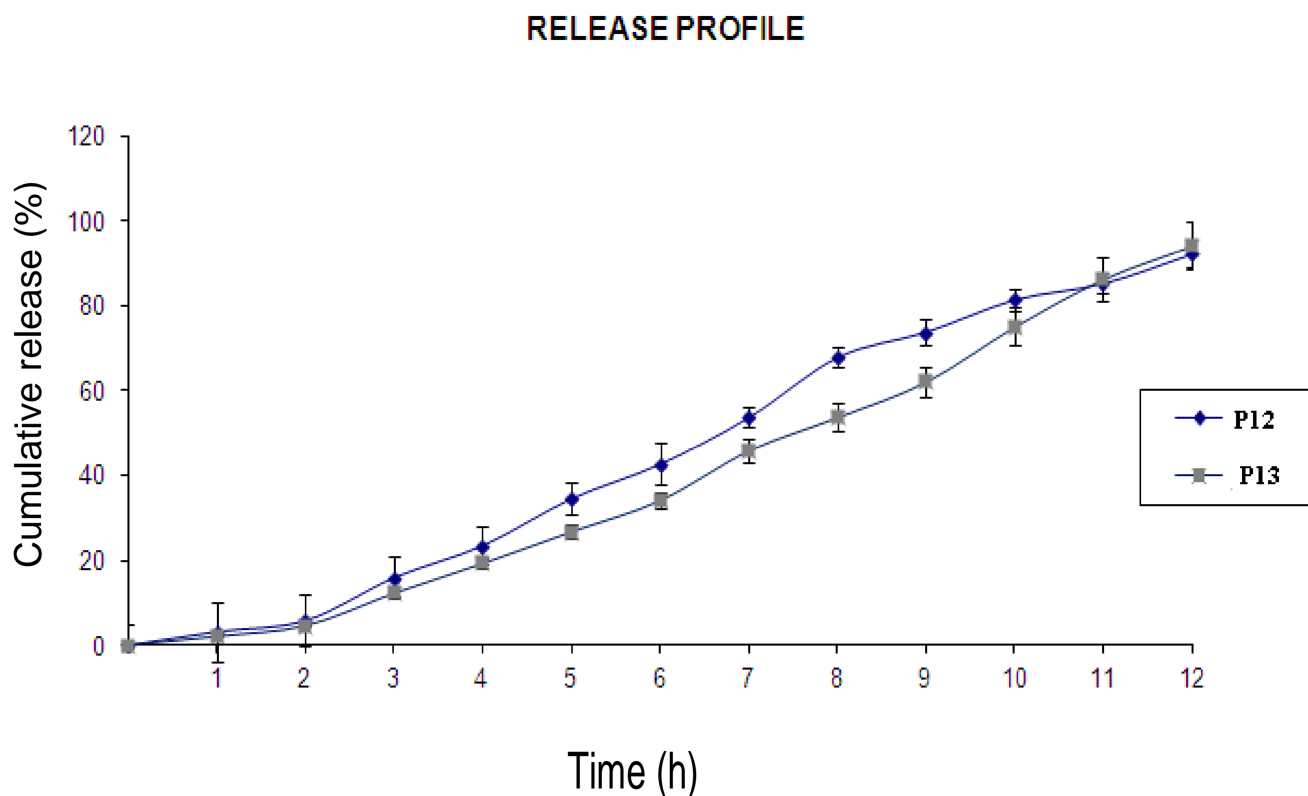

Figure 8. *In vitro* release study.

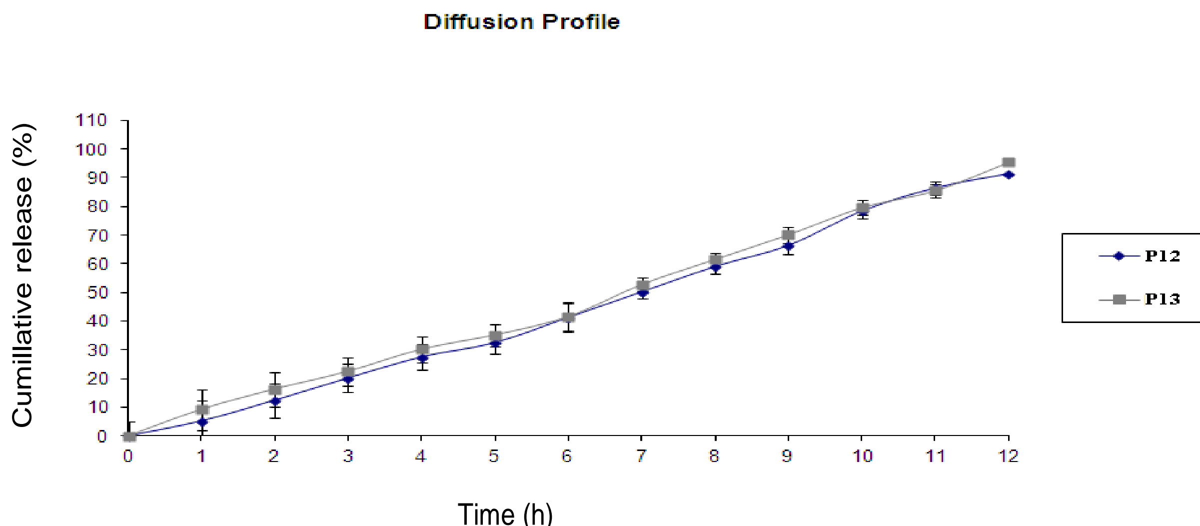

Figure 9. *In vitro* drug permeation studies.

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