The Thai Journal of Pharmaceutical Sciences

Volume 37
Issue 1 *2013*

Article 3

1-1-2013

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Original article

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Abstract:

Verapamil hydrochloride is an antihypertensive agent which undergoes extensive first pass metabolism making it a possible candidate for buccal delivery. Verapamil mucoadhesive buccal patches were prepared using hydroxypropyl methylcellulose, Eudragit RL 100, sodium carboxymethyl cellulose and carbopol. The physicochemical interactions between verapamil and physical mixture were investigated by Fourier transform infrared spectroscopy and differential scanning calorimetry. Results revealed no interaction between drug and polymers. The patches were evaluated for various physicochemical parameters, *in vitro* release studies and *ex vivo* permeation through porcine buccal mucosa. Residual solvent content in patches was determined by gas chromatography and are largely below the tolerated limits. The formulations showed an extended release of the drug upto period of 12 hours during *ex vivo* permeation studies and showed non-Fickian drug release. Stability of the optimized formulation was investigated as per ICH guidelines and was found to be stable with respect to drug content and *ex vivo* permeation.

Keywords: Verapamil; *Ex vivo* permeation; Swelling index; *In vitro* residence time; Bioadhesion; Non-Fickian diffusion

Introduction

Within the oral mucosal cavity, delivery of drugs is classified into three categories: (i) sublingual delivery, which is systemic delivery of drugs through the mucosal membranes lining the floor of the mouth, (ii) buccal delivery, which is drug administration through mucosal membranes lining the cheeks (buccal mucosa), and (iii) local delivery, which is drug delivery into the oral cavity. The buccal route offers certain advantages over other routes of drug administration such as: (i) fast absorption and rapid onset of action, (ii) avoidance of hepatic metabolism by first-pass effect, (iii) better stability of the drug because of avoidance of gastrointestinal drug degradation, (iv) high patient compliance, and finally (v) ease of administration and/or removal from the site of application [1, 2].

To improve buccal delivery of drugs, several new dosage forms have been developed such as tablets/ lozenges (including lyophilized and bioadhesive), laminated systems and patches, hydrogels, ointments, adhesive films [3-5]. However, adhesive patches are preferred over other dosage forms because they are flexible and can be formulated in any shape.

Verapamil is an effective calcium channel blocker, used in the treatment of angina, hypertension and myocardial infarction. It was reported to be rapidly absorbed after oral administration, but undergoes extensive first pass metabolism leading to poor bioavailability (20%). In addition, verapamil has low dose (30 mg), low molecular weight, 491, and lipophilic in nature (log P, 3.28); needed for long term treatment and repetitive dosing [6]. All these parameters make this drug an interesting candidate for buccal administration.

Materials and Methods

Verapamil hydrochloride was a gift from Nicholas Piramil Healthcare Ltd., (Kohir, India). Eudragit RL 100 (Molecular weight: approximately 32000; Viscosity: 1-15 cP) was generous gift from Colorcon Asia PVT Ltd., (Goa, India). Sodium carboxymethyl cellulose (SCMC: molecular weight: approximately 90000; viscosity: 25-50 cP), hydroxypropyl methylcellulose (HPMC K15M: molecular weight: 10000-1500000; viscosity: 15000 cP) were obtained from Tini Pharma Ltd., (Tirupathi, India. Carbopol (viscosity: 29400-39400 cP) was gifted by Inventis drug delivery systems PVT Ltd., (Hyderabad, India). All reagents used were of analytical grade.

Investigation of drug-excipient interactions

Fourier transform infrared spectroscopy

Compatibility between drug and the polymers were studied by FTIR spectra. FTIR studies were carried out for drug and its formulations. The sample was dispersed in KBr powder and pellets were made by applying 6000 kg/cm^2 pressure and analyzed. FTIR spectra were obtained by diffuse reflectance on a FTIR spectrophotometer type FTIR 8400 (Schimadzu Corporation, Japan). The positions of FTIR bands of important functional groups of drug were identified and were cross checked in obtained spectra.

Differential scanning calorimetry (DSC)

DSC studies for drug and its formulations were carried out using DSC-60 calorimeter (Schimadzu Corporation, Japan). The instrument was calibrated with an indium and zinc standard. The sample was heated from 10 to 300°C at a heating rate of 25°C min^{-1} to remove thermal history. The sample was then immediately cooled to 10°C and reheated from 10 to 300°C under the flow of nitrogen at a heating rate of 10°C min^{-1}.

Preparation of patches

Verapamil hydrochloride mucoadhesive buccal patches (Table 1) were prepared by solvent casting technique using chitosan, HPMC K15M, sodium carboxymethyl cellulose (SCMC), Eudragit RL100 and carbopol 934P as polymers. Propylene glycol and dimethylsulphoxide (DMSO) were used as plasticizer and penetration enhancer, respectively. Ethanol, methanol and dichloromethane were used as solvents. Drug was dissolved in little quantity of solvent and polymers were dissolved in remaining solvent/solvent mixture. Drug, polymer solutions along with plasticizer and permeation enhancer were sonicated for 30 min and examined for air entrapment. The solution was poured onto glass moulds of 10 × 5 cm^2 and air dried for overnight at room temperature. An inverted funnel was kept on the

mould for controlled evaporation. The dried film of drug was peeled from the mould and packed in aluminium foil and kept in desiccator till further use.

Backing layer was also prepared by the solvent casting method by dissolving 500 mg ethylcellulose in 15 ml of ethanol-toluene mixture (1:4). The patches were laminated on one side with backing layer and used for the release studies.

Determination of drug content

Drug content of patches was determined by dissolving five patches (1 cm^2) in 100 ml of 6.6 buffer. After suitable dilutions the resultant solution was filtered and analysed for verapamil content spectrophotometrically.

Thickness and weight variation [7, 8]

The thickness of patches was assessed using a micrometer screw age (Mitutoyo, Japan). For each formulation, three randomly selected patches with surface area 1 cm^2 were used. Each patch was weighed individually on an analytical balance (Shimadzu, Japan) and the average weights were calculated.

Folding endurance [9]

Folding endurance of patches was determined manually by repeatedly folding a film at the same place until it ruptureed. The number of foldings required to break or crack a patch was taken as the folding endurance.

Surface pH [10]

Patches were placed in petri dishes containing 5 ml phosphate buffer (pH 6.6) and the pH at the surface was measured by placing the tip of the glass microelectrode of a digital pH meter (Elico LI 120, India) close to the surface of the patch and allowing it to equilibrate for one minute prior to recording. Experiments were performed in triplicate.

Swelling index [11]

Swelling index of the patches was evaluated by placing them in petri dishes containing 4 ml of phosphate buffer pH 6.6 at room temperature. The patches were removed at regular intervals from petri dish and excess buffer was removed using filter paper. The swollen system was reweighed (w_2). The difference between the initial weight (w_1) and the weight gain at regular time interval (w_2) was used to determine swelling index (S.I.) which was calculated as S.I. = $(w_2\text{-}w_1/w_1) \times 100$.

Preparation of porcine buccal mucosa [12]

Buccal tissue was obtained from a local slaughterhouse from freshly sacrificed porcine and used within 3 to 4 hours of sacrifice. The tissue was stored in isotonic phosphate buffer (pH 7.4) at 4°C. Epithelium was separated from the underlying connective tissue using surgical blade and the membrane was used for the experiments.

In vitro residence time [13]

In vitro residence time was determined according to the method described by Nafee *et al.* [10], The apparatus consists of disintegration apparatus with

Table 1 Composition of verapamil buccal patches

Ingredients	Formulation code			
	F1	F2	F3	F4
Verapamil (mg)	500	500	500	500
HPMC K15M (mg)	900	600	600	600
SCMC (mg)	--	300	--	--
Eudragit RL 100 (mg)	--	--	300	--
Carbopol 934P (mg)	--	--	--	300
Dimethylsulphoxide (ml)	0.2	0.2	0.2	0.2
Propylene glycol (ml)	0.4	0.4	0.4	0.4
Ethanol (ml)	--	5	--	5
Methanol (ml)	7.5	5.0	7.5	5.0
Dichloromethane (ml)	7.5	5.0	7.5	5.0

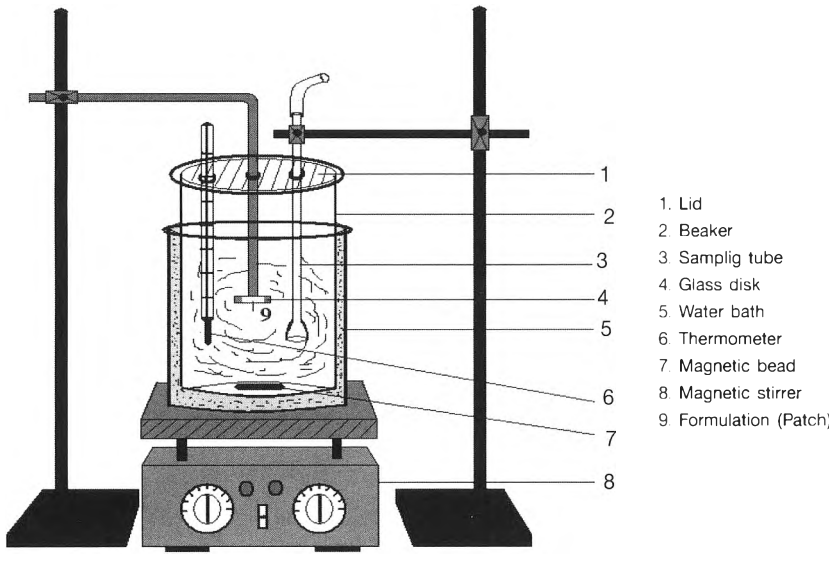

Figure 1 Modified dissolution apparatus used for *in vitro* release study

800 ml of phosphate buffer pH 6.6 maintained at 37 ± 1°C. Porcine buccal mucosa was glued to the glass slide and held vertically in the apparatus. The buccoadhesive patch was hydrated with 0.5 ml of phosphate buffer pH 6.6 and the hydrated surface was brought in contact with the buccal mucosa. The glass slide was 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 required for the complete erosion or detachment of the patch from the mucosal surface was recorded.

***In vitro* mucoadhesion test** [14]

In vitro bioadhesion of the patches was determined using the porcine buccal mucosa. A piece of porcine buccal mucosa was cut and glued with commercially available adhesive on the ground surface of a tissue holder made of thin plastic sheet. Similarly, the patch was glued to another tissue holder of the same size. Then the tissue holders with porcine buccal mucosa and patch were put in contact with each other by applying constant pressure for 5 minutes to facilitate adhesion. The tissue holder with porcine buccal mucosa was allowed to hang on an iron stand with the help of an aluminium wire fastened with the hook provided on the back of the holder. A preweighed lightweight polypropylene bottle was attached to the hook on the back side of the formulation holder with aluminium wire. After a preload time of 5 minutes, water was added to the polypropylene bottle through an intravenous infusion set at a constant rate. The addition of water was stopped when buccoadhesive system was detached from buccal mucosa. The weight required to detach the system from buccal mucosa was noted. The force of adhesion and the bond strength [13] were calculated as;

$$\text{Force of adhesion (N)} = \frac{\text{Weight (gm)}}{1000} \times 9.81 \qquad (1)$$

$$\text{Bond strength (N/m}^2\text{)} = \frac{\text{Force of adhesion (N)}}{\text{Surface area of patch (m}^2\text{)}} \qquad (2)$$

Determination of residual solvents

Methanol [15], ethanol [16] and dichloromethane [17] content in patches was determined by gas phase chromatography on an Agilent 7890 Gas Chromatograph, USA, fitted with a flame ionization detector. For estimation of residual solvents, 1 cm^2 patch was dissolved in little amount of DMSO in a 10 ml volumetric flask and volume was made up to 10 ml with DMSO. The solution was filtered through 0.45 µm filter and degassed using sonicator. From the sample, 1 µl was injected into injection port, the chromatogram was recorded and the peak area of solvent was measured. The concentration of residual solvent was calculated from calibration curve data.

In vitro release studies [14]

The apparatus consists of a receptor compartment (250 ml beaker), which is covered with a thin plastic sheet with three holes, one for a thermometer, second for sample collection tube and third for formulation holding ⊥ shaped glass rod shown in Figure 1. Before starting the *in vitro* study, the patch was attached to glass rod and placed four inches above the receptor. The dissolution medium was 100 ml of phosphate buffer pH 6.6 and the temperature was maintained at 37 ± 1°C on a heat controlled hot plate with a magnetic stirrer. Dissolution fluid was stirred at a constant speed of 50 rpm using a magnetic bead. Samples were withdrawn at regular intervals and the same volume of fresh phosphate buffer pH 6.6 was replaced into the beaker to maintain sink condition. The samples were filtered through a 0.45 µm filter paper (Millipore) and drug concentration was analyzed spectrophotometrically.

HPLC analysis [18]

Analysis of samples was performed using a Schimadzu 10 AVP (Japan) HPLC system equipped with UV detector and Waters C-18 column (300 x 4.6 mm i.d) at ambient temperature. The mobile phase was a mixture of 700 ml of phthalate buffer pH 2.8 and 250 ml of acetonitrile. The solution was filtered through 0.45 µm filter and degassed by sonication. The flow rate was 1 ml per minute. Detection was carried on at 275 nm wavelength. A calibration curve was plotted for verapamil in the range of 10-50 µg ml-1. A good linear relationship was observed between the concentration of verapamil and its peak area (r^2 = 0.9972). Precision and accuracy of the HPLC method were estimated.

Ex vivo permeation studies [19]

One-cm^2 patch under study was placed in intimate contact with the excised porcine buccal mucosa and mounted between the two compartments of Franz diffusion cell. A teflon bead was placed in the receptor compartment filled with 25 ml of pH 6.6 phosphate buffer. The diffusion cell was thermostated at 37 ± 1°C and at a rate of 50 rpm. The samples were withdrawn at regular intervals and the same volume of fresh phosphate buffer pH 6.6 was replaced into the diffusion cell to maintain sink condition. Samples were filtered through a 0.45 µm filter paper (Millipore) and analyzed for drug content using HPLC and the data was statistically analysed by one-way ANOVA followed by Turkey post-hoc test for multiple comparison using GraphPad Prism software program. Differences were considered to be significant at a level of $p < 0.05$. The permeability coefficients (P) were calculated as follows [20]:

$$P = (dQ/dt)/(CA) \quad (3)$$

where,

dQ/dt = permeation rate

C = concentration of the donor chamber

A = surface area of diffusion

Steady state fluxes (J_{ss}) were calculated by dividing the slope of cumulative amount permeated Vs time curve by the diffusional area.

Drug release from backing layer [21]

One-cm^2 patch was placed between the two compartments of a Franz diffusion cell at 37 ± 1°C with the backing layer facing the receptor compartment filled with 25 ml of pH 6.6 phosphate buffer. The samples were withdrawn at regular intervals and the same volume of fresh phosphate buffer pH 6.6 was replaced into the diffusion cell to maintain sink condition. Samples were filtered and analyzed for drug content.

Stability in artificial saliva [22, 23]

Stability of the patches was assessed in artificial saliva. Patches were placed in petri dishes containing 5 ml of artificial saliva and kept in a temperature controlled oven at 37 ± 1°C for 6 hours. The patches were examined for changes in texture and drug content.

Stability studies [24]

Stability studies were conducted according to the ICH Q1A (R2) guidelines. Patches were wrapped in aluminum foil and were kept in stability chamber at a temperature of 40 ± 2°C and 75 ± 5% RH for 6 months. Samples were withdrawn at the end of 6 months and

analyzed for drug content and ex vivo permeation through porcine buccal mucosa. Zero time samples were used as control for the study and the results were statistically analyzed by using *t*-test and $p < 0.05$ were considered as significant.

Results and Discussion

Investigation of drug-excipient interactions

FTIR spectral analysis

Verapamil pure drug and its formulations were subjected to FTIR spectroscopic analysis. The obtained spectra are shown in Figure 2. The FTIR spectra of pure verapamil showed sharp characteristic peaks at 1261 (C-O stretch), 1591, 1518 (bands due to skeletal vibrations of the benzene ring), 2235 (C=N stretch), 2578, 2542, 2453 (N-H stretch) and 2839, 2956, 2937 cm^{-1} (C-H stretch). All the above characteristic peaks appeared in the spectra of formulations (F1-F4) at the same wavenumbers indicating no modification or interaction between the drug and polymers.

Differential scanning calorimetry

DSC studies were carried out for verapamil pure drug, physical mixture and its formulations. DSC thermograms of verapamil and its formulations are

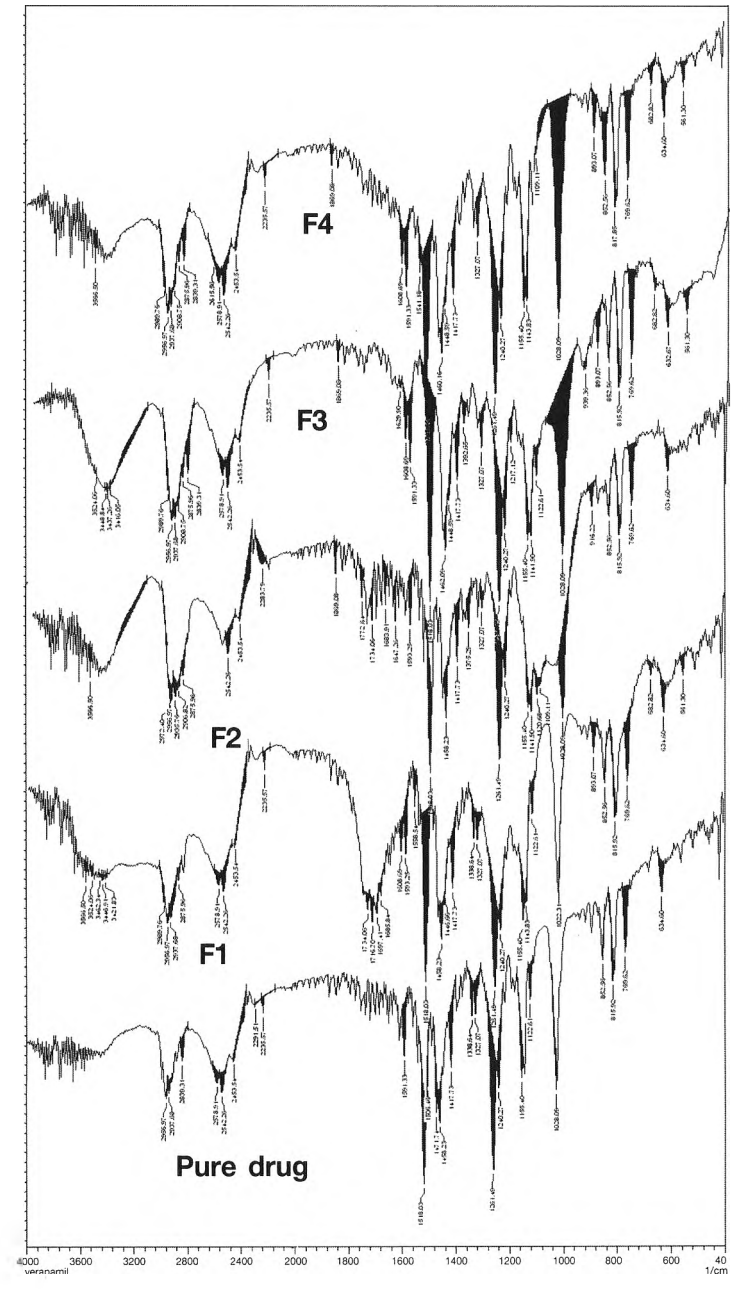

Figure 2 FTIR spectra of verapamil and its formulations (F1-F4)

shown in Figure 3. The melting point of verapamil is 140-144°C. The DSC thermogram of verapamil showed an endothermic peak at 144.32°C corresponding to its melting temperature, which was also detected in the thermograms of formulation, signifying no change in crystal form and interaction between the polymers.

Physicochemical evaluation of verapamil buccal patches

The % drug content of patches was found to be 98.27 ± 1.37, 99.50 ± 1.51, 98.63 ± 0.76 and 99.33 ± 1.56 for the formulations F1, F2, F3 and F4 respectively (Table 2). The weight of the patches was found to be 27.22 ± 0.35 mg, 26.09 ± 0.18 mg, 27.28 ± 0.47 mg and 26.28 ± 0.16 mg for the formulations F1, F2, F3 and F4 respectively. The thickness was found to be 291.00 ± 2.08 µm, 258.00 ± 1.52 µm, 253.00 ± 2.00 µm and 271.00 ± 2.51 µm for the formulations F1, F2, F3 and F4 respectively. The mean folding endurance values were found to be 231.00 ± 9.16, 243.00 ± 7.02, 299.00 ± 2.51 and 239.00 ± 7.50 for the formulations F1, F2, F3 and F4 respectively. Folding endurance of the patches was in the order F3>F2>F4>F1. F3 showed maximum folding endurance may be due to the presence of Eudragit [25-27]. The folding endurance of all the patches was optimum, the patches exhibited good physical and mechanical properties.

High alkaline or acidic pH of patches may cause irritation to the buccal mucosa and influence the degree of hydration of polymers [28, 29], so the surface pH of patches was determined to optimize release and adhesion. The surface pH of all formulations was in the pH range 6-7, i.e close to buccal pH. Swelling index of the patches was in the order F2>F1>F4>F3 and showed

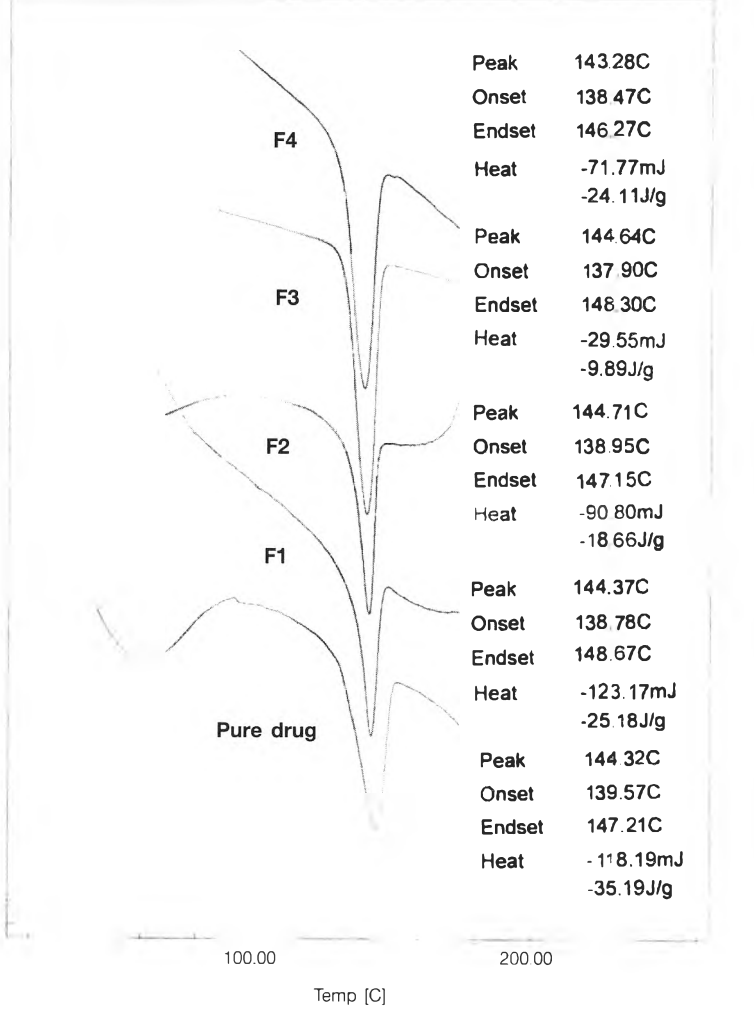

Figure 3 DSC thermograms of verapamil and its formulations (F1-F4)

significant difference. Formulation F2 showed higher percentage of swelling may be due to the presence of HPMC and SCMC. This may be due to more hydrophilic nature of SCMC and presence of hydroxyl groups in SCMC [30]. Formulation F3 showed less percentage of swelling due to the presence of Eudragit, a hydrophobic polymer [31, 32].

In vitro residence time of the patches was in the following order F4>F2>F1>F3 and showed significant difference. Higher residence time in F4 may be due to the presence of carbopol, a good mucoadhesive polymer. Addition of carbopol and SCMC were found to maximize the residence time which may be due to formation of strong hydrogen bond with the cell surface and creation of strong bioadhesion. Addition of Eudragit RL100 leads to decrease in the residence time of the patches which may be due to poor solubility of Eudragit. The results were in agreement with earlier reports [33]. An effective buccal mucosal delivery device must maintain intimate contact with mucus layer overlying the epithelial tissue. This parameter is critical for successful utilization of these dosage forms. Hence, *in vitro* mucoadhesion testing was carried by using pork mucosal membrane, which gives indirect measurement of the bioadhesive strength in grams. The bond strength values were found to be 804.29, 946.08, 763.87 and 1076.81 N/m^2 for F1, F2, F3 and F4 respectively and significant difference was found in bioadhesive strength of patches (Table 3).

Many hydrophilic polymers adhere to mucosal surfaces as they attract water from the mucus gel layer adherent to the epithelial surface. This is the simplest mechanism of adhesion and has been defined as "adhesion by hydration". Various kinds of adhesive force, e.g. hydrogen bonding between the adherent polymer and the substrate, i.e. mucus, are involved in mucoadhesion at the molecular level [28, 29, 34]. HPMC,

Table 2 Physical evaluation of verapamil buccal patches

Formulation	Parameter						
	% Drug content	Weight variation (mg)	Thickness (μm)	Folding endurance	Surface pH	Swelling index	*In vitro* residence time (min)
F1	98.27 ± 1.37	27.22 ± 0.35	291.00 ± 2.08	231.00 ± 9.16 (c*)	6.30 ± 0.26 (c*, d*)	51.54 ± 3.55 (c*, d*)	273.00 ± 4.51 (b*, c*, d*)
F2	99.50 ± 1.51	26.09 ± 0.18	258.00 ± 1.52	243.00 ± 7.02 (c*)	6.73 ± 0.10	55.53 ± 1.26 (c*, d*)	292.00 ± 5.51 (a*, d*)
F3	98.63 ± 0.76	27.28 ± 0.47	253.00 ± 2.00	299.00 ± 2.51 (a*, b*, d*)	6.82 ± 0.09 (a*)	23.67 ± 0.86 (a*, b*, d*)	224.00 ± 5.13 (a*, d*)
F4	99.33 ± 1.56	26.28 ± 0.16	271.00 ± 2.51	239.00 ± 7.50 (c*)	6.80 ± 0.16 (a*)	43.63 ± 1.49 (a*, b*, c*)	323.00 ± 5.29 (a*, b*, c*)

Mean ± SD, n = 3. a/b/c/d: significantly different from F1/F2/F3/F4 respectively, *: $p<0.05$

Table 3 *In vitro* bioadhesive strength of verapamil patches

Formulation	Bioadhesive strength		
	Weight (gm)	Force of adhesion (N)	Bond strength (N/m^2)
F1	8.19 ± 0.41 (d*)	0.0804	804.29
F2	9.64 ± 0.71	0.0946	946.08
F3	7.78 ± 0.39 (d*)	0.0763	763.87
F4	10.98 ± 1.08 (a*, c*)	0.1076	1076.81

Mean ± SD, n = 3. a/c/d: significantly different from F1/F3/ F4, *: $p<0.05$

carbopol and SCMC polymers have been demonstrated to create a tenacious bond with the mucus membrane resulting in strong bioadhesion. Formulation F4 showed greater bioadhesive strength followed by F1, F2 and F3. Addition of carbopol and SCMC were found to maximize [34], where as addition of Eudragit RL-100 was found to minimize the bioadhesive property of patches due to poor hydration [25].

According to ICH guidelines ethanol is a class III solvent (solvents with low toxic potential) and the limit of 5000 ppm is acceptable without justification. Methanol, dichloromethane and toluene are class II solvents (solvents to be limited) thus the limits of 3000 ppm, 600 ppm and 890 ppm respectively are acceptable. The residual solvents content in verapamil patches are largely below the tolerated limits which are given in Table 4.

In vitro release studies

In vitro release studies of patches were carried out in triplicate and after 6 hours, the release was found to be 81.15 ± 1.97, 91.42 ± 1.74, 71.72 ± 2.59 and 84.82 ± 1.79% for the formulations F1, F2, F3 and F4 respectively (Figure 4). The data of *in vitro* release was analyzed by one way ANOVA and significant difference was observed between the means. *In vitro* release studies clearly showed that the percent release of verapamil was maximum i.e., 91.42 ± 1.74% from the buccal patch F2. The order of drug release was found to be F2>F4>F1>F3. F3 showed less release due to its low swelling index.

Formulation containing SCMC (F2) showed maximum swelling and a gel layer was formed on the surface which may be due to more hydrophilic nature of SCMC. When swelling is prevalent, drug diffusion may occur through the solvent-filled pathways of swollen patch. Erosion of polymer matrix can also affect the drug release [35]. The loosely bound polymer molecules in these patches eroded readily, allowing faster release of verapamil from the patches [36]. Though the formulation F1 showed greater swelling index than formulation F4 and slower release of the drug which may be due to the high viscous nature of HPMC K15M. In addition, HPMC K15M forms a thick gel (diffusion path length) that acts as a barrier for drug diffusion and prevents matrix disintegration and additional water penetration [37].

Formulation F4 showed faster release, which may be due to presence of carbopol and HPMC. Carbopol having tendency to undergo ionization at pH 6.8 and activates negative charges at the backbone of polymer. Repulsion between these like charges leads to uncoiling of the polymer to produce an extended structure capable of greater uptake of water [21, 38]. Thus owing to generation of pores by HPMC and uncoiling of carbopol at pH 6.8, the system absorbs more water and there by promotes diffusion, which in turn leads to an increase in release of drug. Presence of Eudragit in formulation F3 slower the drug release, which may be due to water insolubility of Eudragit, lower dissolution and slower erosion of films [32].

Ex vivo permeation studies

Ex vivo permeation studies for the patches were carried out in triplicate and after 12 hours the release was found to be 74.91 ± 7.01, 90.63 ± 6.21, 64.71 ± 6.71

Table 4 Residual solvent content in verapamil patches

Residual solvent	Limit (ppm)*	Concentration (ppm)			
		F1	F2	F3	F4
Ethanol	5000	---	69.90 ± 6.25	---	72.16 ± 5.26
Methanol	3000	38.90 ± 3.99	44.94 ± 4.12	42.74 ± 3.90	43.77 ± 3.62
Dichloro-methane	600	14.05 ± 4.78	15.37 ± 3.89	16.84 ± 3.98	14.78 ± 4.48

Mean ± SD, n = 3, *As per ICH guidelines

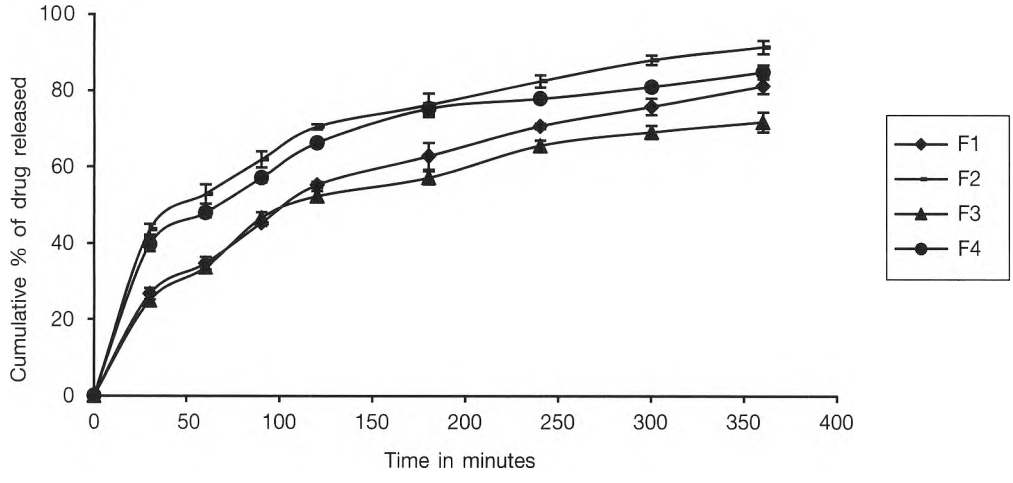

Figure 4 *In vitro* drug release profile of verapamil patches

Table 5 Correlation coefficient (r^2) and rate constant of different kinetic models for verapamil patches

Formulation	n value	Correlation coefficient (r^2)				Drug transport mechanism
		Zero order	First order	Higuchi	Peppas	
F1	0.9098	0.9937	0.9651	0.9555	0.9941	Non-Fickian diffusion
F2	0.8317	0.9529	0.9869	0.9790	0.9904	Non-Fickian diffusion
F3	0.9440	0.9970	0.9768	0.9593	0.9956	Non-Fickian diffusion
F4	0.8641	0.9927	0.9663	0.9601	0.9917	Non-Fickian diffusion

and 81.62 ± 4.81% for the formulations F1, F2, F3 and F4 respectively (Figure 5). The data of *ex vivo* permeation was analyzed by one way ANOVA and significant difference was observed between the means. In *ex vivo* permeation study formulation F2 showed a maximum release of the drug, 90.63 ± 6.21% in 720 minutes, and this formulation was considered as optimized one and used for further study. The order of drug release was F2>F4>F1>F3.

The drug release data obtained were fitted into zero order, first order, Higuchi and Korsmeyer-Peppas equations to know the mechanism of drug release from these formulations. The *ex vivo* permeation profile of formulations F1 and F2 could be best expressed by Korsmeyer-Peppas model, as the plots showed highest linearity (r^2=0.9941, 0.9904, respectively). Formulations F3 and F4 formulation could be best expressed by zero order model, as the plots showed highest linearity (r^2= 0.9970, 0.9927, respectively). All the formulations showed a non-Fickian release pattern as it was evidenced from the release exponent (n > 0.5) (Table 5). This indicates coupling of the diffusion and erosion mechanism, called anomalous diffusion and shows that the drug release is controlled by more than one process. So, the suggested drug release mechanism for verapamil patches may be combination of diffusion and erosion of polymer matrix.

The time taken for the permeation of 50% verapamil was found to be 446, 282, 552 and 398 minutes for the formulations F1, F2, F3 and F4 respectively. The mean steady state flux (J_{ss}) was found to be 0.62 ± 0.04, 0.77 ± 0.05, 0.54 ± 0.02, 0.70 ± 0.05 mg/cm^2/hr and the permeability coefficient was found to be 0.062 ± 0.004, 0.077 ± 0.010, 0.054 ± 0.002, 0.070 ± 0.010 cm/hr

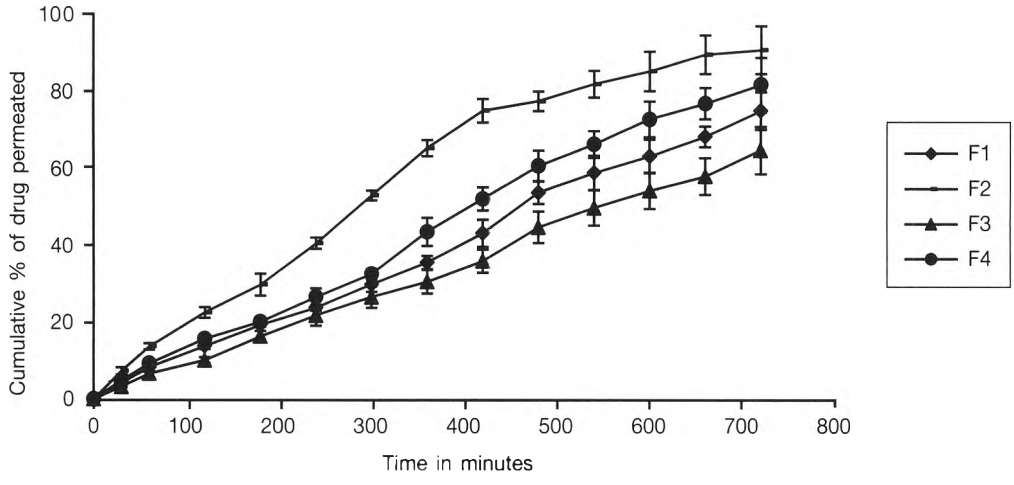

Figure 5 *Ex vivo* permeation profile of verapamil patches (F1-F4)

Table 6 *Ex vivo* permeation flux and permeability coefficient of verapamil buccal patches

Formulation	$t_{50\%}$ (min)	Flux (J_{SS}) (mg/cm^2/hr)	Permeability coefficient (cm/hr)
F1	446.00 ± 33.89	0.62 ± 0.04	0.062 ± 0.004
	(b*)	(b*)	(b*)
F2	282.00 ± 19.45	0.77 ± 0.05	0.077 ± 0.010
	(a*, c*, d*)	(a*, c*)	(a*, c*)
F3	552.00 ± 16.22	0.54 ± 0.02	0.054 ± 0.002
	(b*, d*)	(b*, d*)	(b*, d*)
F4	398.00 ± 21.28	0.70 ± 0.05	0.070 ± 0.010
	(b*, c*)	(c*)	(c*)

$t_{50\%}$: Time required for 50% of verapamil to be permeated.
a/b/c/d: significantly different from F1/F2/F3/F4 respectively, *: $p<0.05$.

(Table 6) for the formulations F1, F2, F3 and F4 respectively.

Drug release from backing layer was investigated, to ascertain the efficiency of backing membrane to provide unidirectional release of drug through the patch. Results of the study showed that no drug was released during the study. This indicated that backing layer of ethyl cellulose was able to prevent the release of drug.

Stability studies

Stability study of F2 was conducted in artificial saliva to mimic the stability of drug and the formulation in the oral cavity. No color change was observed. Thickness of patches increased to 19.61% owing to swelling in artificial saliva over 6 hours. The recovery of drug from the patches was 99.5% (9.91 mg) indicating maximum utilization of the drug incorporated.

Accelerated stability studies were performed for optimized formulation (F2) as per ICH Q1A (R2) guidelines at 40 ± 2°C and 75 ± 5% RH for 6 months. After specified duration, visual examination of the buccal patches did not show any change in morphology. The results of the stability studies revealed that there were no significant changes in drug content and *ex vivo* permeation through porcine buccal mucosa. Shelf life of the formulation was calculated by using "Stab R" software [39] and it was found to be 28 months. The cumulative percentage of verapamil permeated in 12 hours was found to be 84.93 ± 3.56%. Flux and permeability coefficient of verapamil was found to be 0.72 ± 0.05 mg/cm^2/hr and 0.07 ± 0.01 cm/hr respectively for the optimized formulation after stability study.

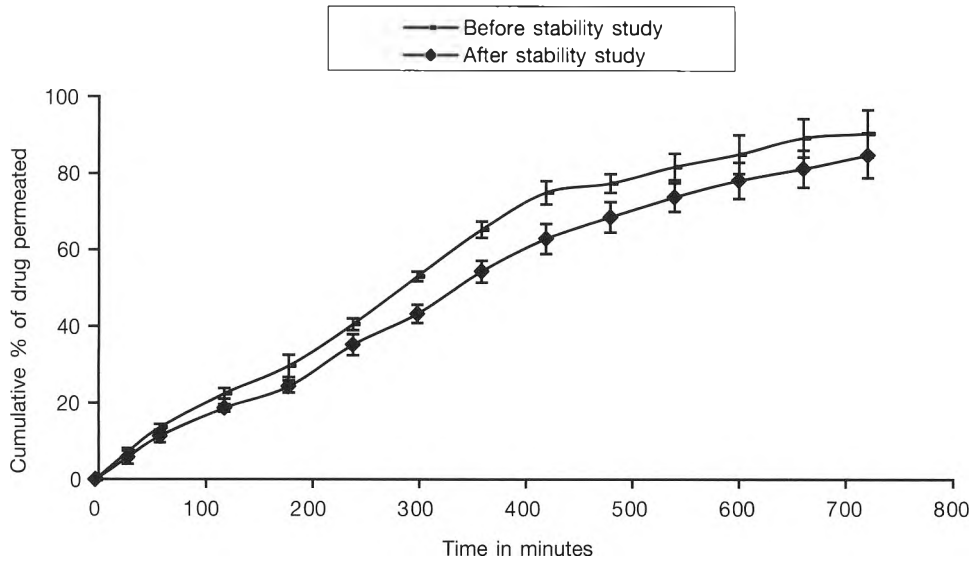

Figure 6 *Ex vivo* drug permeation profile of F2

The *ex vivo* permeation profile of F2 after stability study could be best expressed by Korsmeyer-Peppas model, as the plots showed highest linearity (r^2, 0.9944) and the obtained release exponent (n) value, 0.8674, supported non-Fickian release (Figure 6). It was observed that there was no change in the best fit model and transport mechanism even after stability study.

Conclusion

The present study indicates enormous potential of mucoadhesive buccal patches containing verapamil for systemic delivery with an added advantage of circumventing the hepatic first pass metabolism. HPMC can be used as a bioadhesive polymer for buccal delivery resulting in patches with favorable film properties and optimum bioadhesion sufficient to retain the dosage form until the drug is permeated. Good results were obtained both *in vitro* and *in vivo* conditions for patches. The statistical investigation of *ex vivo* permeation data showed that the coupling of the diffusion and erosion is the mechanism of drug release. From the present investigation, it can be concluded that such buccal patches of verapamil may provide sustained buccal delivery for prolonged periods in the management of hypertension, which can be a good way to bypass the extensive hepatic first-pass metabolism.

Acknowledgements

The authors are thankful to M/s Nicholas Piramil Healthcare Ltd., Kohir, India for providing gift sample of verapamil hydrochloride. The authors are thankful for MNR Educational Trust, Hyderabad for providing support in carrying out the research work.

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