Design and *In vivo* Evaluation of Buccoadhesive Hydrophilic Polymer Matrix Films of Losartan Potassium

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ABSTRACT

Introduction: The buccal mucosal route can be considered as an alternative to oral administration for the purpose of improving bioavailability and therapeutic efficacy of drugs such as losartan potassium that suffers from significant first pass metabolism. Objectives: The objective of this investigation was to formulate mucoadhesive buccal films of losartan potassium, an antihypertensive and evaluate them for in vitro drug release and in vivo buccal absorption. Methods: Film formulations were prepared from the hydrophilic polymers, hydroxy propylmethyl cellulose/sodium carboxymethyl cellulose and carbopol 934 P with suitable plasticizers. They were then subjected to studies for evaluating physical and mechanical properties as well as in vitro and in vivo drug release in rabbits. Results: Formulations containing higher proportions of the cellulose polymers exhibited faster rate of swelling. Faster swelling due to rapid rehydration and greater bioadhesive strength was produced by the sodium carboxymethyl cellulose films in comparison to those of hydroxyl propylmethyl cellulose. Kinetic analysis of in vitro drug release data indicated first order release from all formulations and application of Peppas equation indicated non-fickian diffusion for most formulations. Plasma level studies in rabbits revealed that the calculated values of the pharmacokinetic parameters $C_{_{max}}$, $T_{_{max}}$ and AUC in the groups that received the optimized formulations were statistically different (P<0.005) from that of the groups that received the oral solution at the same dosage level. The differences in these parameters between F1 and F4 were however, not statistically significant. Conclusion: In vivo studies have confirmed the efficacy of the buccal route in improving the systemic bioavailability of losartan potassium from mucoadhesive films. F1 and F4 can be considered as promising formulations for clinical application.

Key words: Buccal administration, Carbopol, Losartan, Mucoadhesive, Rabbits, Sodium carboxymethyl cellulose.

INTRODUCTION

The oral route may present several problems with respect to systemic administration of some drugs such as enzyme inactivation in the gastro intestinal (GI) tract, erratic or poor absorption due to solubility issues, degradation by GI pH and first pass metabolism. The oral mucosa is highly permeable to many drugs and together with a rich vasculature would ensure rapid drug absorption which makes the buccal route an attractive alternative to the oral and parenteral routes for systemic drug delivery. Buccal mucosal delivery would be able to avoid most of the complications associated with the oral route and could help to improve their bioavailability.¹ Unlike the sublingual site, the buccal mucosa permits prolonged retention of a dosage form especially with the use of mucoadhesive polymers without interference in speech or mastication. Therefore prolonged release of the incorporated drug in such dosage forms would be within their scope.² Many mucoadhesive polymers such as hydroxy propyl methyl cellulose (HPMC) are also capable of prolonging the release of the entrapped active ingredient by virtue of their swelling effect as a result of hydration.³ Different oral mucoadhesive dosage forms have been developed such as tablets, gels, patches and films which make use of

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polymers such as carbopols and HPMC that prolong their residence time in the oral cavity. However, buccal films or patches are preferred to tablets since the former are more flexible, comfortable to use and allow for better adhesion of the system to the oral mucosa.⁴ The antihypertensive, losartan potassium is an angiotensin II receptor (type AT₁) antagonist and on oral administration is reported to be well absorbed but subjected to extensive first-pass metabolism by cytochrome P450 enzymes, the systemic bioavailability of losartan being approximately 33%.5 The biological halflife of losartan is 2 h and requires frequent administration in the control of hypertension. Typically, losartan potassium is orally administered as 25 mg tablets once or twice a day with total daily dosage ranging from 25 to 100 mg. Administration of this drug as mucoadhesive buccal films would not only prolong its release thereby allowing for reduced frequency of administration and improving patient compliance but may also help to increase the systemic availability by avoiding first pass metabolism. The objective of this study is to formulate mucoadhesive buccal films of losartan potassium with the intention of prolonging drug release and enhancing bioavailabilty as an alternative to the conventional oral tablets in the control and treatment of hypertension.

MATERIALS AND METHODS

Materials

Losartan Potassium was generously gifted to us by Sun Pharmaceutical Industries Ltd. Vapi, Gujarat. Sodium carboxymethyl cellulose high viscosity grade (SCMC), hydroxy propylmethyl cellulose K 15 M (HPMC)), carbopol 934P (CP) were procured from Merck India Ltd., Mumbai. Ethyl cellulose (Ethocel 10, standard premium) and Polyethylene Glycol 400 (PEG 400) was obtained from Dow chemicals and CDH, New Delhi respectively. Organic solvents used were of analytical grade and other chemicals of laboratory grade.

Preparation of drug loaded films

Drug loaded films in different ratios of SCMC/HPMC to Carbopol 934(CP) and constant proportion of PEG 400 were prepared by solvent casting technique. PEG 400 acts as the plasticizer. The polymers, HPMC or SCMC, PEG 400 and CP were taken in the ratios of 0.7:1.0:0.3, 0.5:1.0:0.5 and 0.3:1.0:0.7. The total polymer concentration used was 1% w/v and the solution was allowed to stir for 6 h.³ To incorporate the drug, losar-tan potassium was dissolved in 5 ml of distilled water and the solution was mixed with the gel. Since CP is acidic, the gel solution was adjusted to slightly alkaline

pH of 6.5-7.5 with 18% w/v sodium hydroxide solution before adding the drug to avoid incompatibility. The gel was allowed to stand overnight for de-aeration and then cast onto a circular glass mould of 9 cm diameter and dried in the oven at 45°C until completely dry. The films were carefully removed from the mould and observed for any imperfections. Circular films of 15 mm diameter were punched out so that each contained 10 mg of the drug. The films were carefully wrapped in aluminum foil and enclosed in a container stored at room temperature and 58% relative humidity until further use. This storage condition helped to maintain the elasticity and integrity of the films. The composition of the films is given in (Table 1).

Evaluation of films for physical characteristics Uniformity of weight and thickness

The individual weight each of 10 samples of each formulation was determined. The average weight was calculated as shown in (Table 2).

The thickness of each of 10 films of each type of formulation was measured using a micrometer screw gauge and the average was determined as shown in (Table 2).

Uniformity of drug content

This parameter was determined by dissolving one film of 15 mm diameter designed to contain 10 mg of losartan potassium by homogenization in 50 ml of simulated saliva of pH 6.8 for 5 h with occasional shaking. About 5 ml of the solution was taken and filtered and 1 ml of the filtrate was diluted to 10 ml with the same solvent. The absorbance was measured at 250 nm using an UV spectrophotometer. The experiments were carried out in triplicate for the films of all formulations and average values were recorded.

Surface pH

The surface pH of the films was measured in order to determine their irritation potential of the films to the buccal mucosa due to change in pH *in viva*. The film to be tested was placed in a Petri dish and was moistened with 0.5 ml of distilled water and kept for 1 h. The pH was noted after bringing the electrode of the pH meter in contact with the surface of the formulation and allowing equilibration for 1.0 min.⁶

Folding Endurance

The folding endurance is a measure of the mechanical strength and flexibility of the films necessary for handling. This property was determined by mechanically folding one patch at the same place repeatedly till it broke or at least up to 300 times which is considered suitable for revealing satisfactory film properties. The number of times the film could be folded at the same place without breaking gives the value of the folding endurance.^{7,8}

Tensile Strength Measurement

Tensile Strength of the films was evaluated using the Instron universal testing instrument (Model 1121, Instron Ltd., Japan, NITK, Suratkal) with a 5-kilogram load cell. The freshly prepared films free from air bubbles or physical imperfections were cut into strips of 10 cm length and 1 cm wide. One such strip was held between two clamps positioned at a distance of 3 cm apart. The strip was pulled by the top clamp slowly at a rate of 100 mm/min until it broke. This force at break and the resulting elongation produced when the film was pulled were measured. Films which broke at and not between the clamps were not included in the calculations. The measurement was repeated thrice for each formulation.

The Tensile strength and Percentage elongation are two mechanical properties that were evaluated during this test. Tensile strength is the maximum stress applied to a point at which the film sample breaks and can be calculated from the applied load at break and cross sectional area of fractured film as a mean of three measurements from the following equation.⁴

Force at break

Tensile strength =

Initial cross sectional area of the sample (mm²)

Percentage elongation can be obtained by following equation:

% Elongation at break = Original length

Measurement of Swelling Index

The studies for Swelling Index of the films were conducted in simulated salivary fluid of pH 6.75. The film sample (surface area: 1.75 cm^2) was weighed and placed in a previously weighed stainless steel wire sieve of approximately 800 µm mesh. The mesh containing the film sample was immersed in 20 ml of the simulated salivary medium contained in a porcelain dish. At definite time intervals, the stainless steel mesh was removed, excess moisture removed by carefully wiping with absorbent tissue and reweighed. Increase in weight of the film was determined at each time interval until a constant weight was observed. The degree of swelling was calculated using the formula:

$$S.I = (W_t - W_0) / W_0$$

Where S.I is the Swelling Index, W_t is the weight of film at time t and W_0 is the weight of the film at time 0.⁴

Measurement of bioadhesive force

The tensile strength required to detach the bioadhesive film from the mucosal surface was applied as a measure of the bioadhesive performance. In the present work a specially fabricated assembly based on published literature was used.⁷ Porcine cheek pouch was used as the model surface for bioadhesion testing. After the cheek pouch was excised and trimmed evenly, it was then washed in simulated salivary fluid and then used immediately. The working of a double beam physical balance formed the basis of the bioadhesion test assembly.

The left pan was removed and hung with a stainless steel chain. A Teflon block with 1.5 inches height and 1.5 inches diameter was hung with the stainless steel chain to balance the weight of the other pan. The height of the total set up was adjusted to accommodate a glass container or beaker below it leaving a head space of about 0.5 cm in between. Another Teflon block of 2 inches height and 1.5 inches diameter was kept inside the glass vessel, which was then positioned below the top hung Teflon block. Suitable weights were added (15.0 g) on the right pan to balance the beam of the balance. The porcine cheek membrane was attached with the mucosal side upward onto the lower Teflon block which was then placed in the glass vessel. Sufficient simulated salivary fluid was filled into the beaker so that the surface of the fluid just touches the mucosal surface to keep it moist. The beaker was positioned below the upper Teflon block. The film under test was fixed to the surface of the upper block with glue. The 15.0 g weight on the right pan was removed and this lowers the upper Teflon block with film, so that it is in contact with mucosal surface. A load of 20.0 g was placed as initial pressure on the upper block for 3 min. Slowly weights were added onto the right pan starting from 500 mg at 30 sec time intervals. The total weight at which detachment of the film from the mucosal surface takes place is noted and the bio-adhesion force was calculated per unit area of the patch as follows:

$F = (W_w \times G)/A$

Where F is the bioadhesion force $(kg/m/s^2)$, W_w is the mass applied (g), G is the acceleration due to gravity (cm/s^2) and A is the surface area of the patch (cm^2) .

Ex vivo mucoadhesion time/ residence time

The success of the formulation as a buccal film for systemic delivery depends on how long it is able to adhere or reside at the site of application on the mucosa before it dissolves or erodes away. This is particularly important for films meant for sustained release of the drug, where it is desirable that the film remains until most of the drug is released. The residence time for the formulation on the buccal mucosa was determined by observing the time taken for the film to detach or erode completely from the surface of freshly excised porcine buccal mucosa onto which the film was applied. The porcine tissues were fixed on the internal side of a beaker with cyano acrylate glue. The film was wetted with 50 µl of simulated saliva fluid and was pasted to the porcine buccal tissue by applying a light force with a finger tip for 20 sec. The beaker was filled with 200 ml simulated saliva fluid and kept at 37°C. After 2 min a 50 rpm stirring rate was applied to simulate the buccal cavity environment and during the test, the time taken for the film to completely erode or detach from the mucosa was observed as the ex vivo mucoadhesion time.9

In vitro drug release studies

In vitro release studies were carried out by a slight modification of the method suggested by Perioli L., et al and Ilango et al.^{9,10} A buccal film was attached to the wall of the dissolution vessel such as a 250 ml beaker, midway from the bottom with instant adhesive or cyanoacrylate glue. After 2 min, the vessel was filled with 200 ml of simulated saliva of pH 6.8 and placed on a magnetic stirrer. The temperature of the dissolution medium was maintained at $37 \pm 0.5^{\circ}$ C and stirred at 50 rpm. At predetermined time intervals 5 ml samples were withdrawn and replaced with fresh medium. The samples were diluted appropriately with simulated saliva and assayed spectro photometrically at 250 nm. Four film samples of each formulation were subjected to drug release studies in this manner and the average cumulative percentage drug released was determined.

In vivo buccal permeation studies of drugs from mucoadhesive films in rabbits

Based on *in vitro* mucoadhesion and drug release studies, the optimized formulations were selected from these studies and backed with ethyl cellulose films to ensure one way flux of the drug during use.

The *in vivo* pharmacokinetic study in rabbits was conducted after obtaining approval from the Institutions Animal Ethics Committee of the K.S. Hegde Medical Academy, Derelakatte, Mangalore. New Zealand white rabbits of either sex and body weight of 2.5-3.0 kg were

used for the test. To carry out the study each formulation was applied to the buccal mucosa of the anaesthetized rabbits. Before the test rabbits were fasted overnight with ad libitum, having stored them in individual cages for acclimatization period of one week before the experiment was carried out. The rabbits were divided into three groups of four each. The rabbits were weighed and anaesthetized by an intramuscular injection of ketamine HCl (40 mg/Kg) and Xylazine (10 mg/Kg).^{11,12} Rabbits remain anaesthetized for 4 h without respiratory depression when an additional dose of the anaesthetic combination was administered after 11/2 h. After 10 min of initiation of anaesthesia, aqueous solutions of the drug containing the same amount of drug as the films were administered orally through a plastic tube to the first group. The selected buccal patches containing the drug were moistened with 30 µl of simulated saliva of pH 6.75 and applied to the buccal pouch of the second and third group of rabbits. Before use, the cannula and blood collection tubes were rinsed with 3.8% sodium citrate solution as anti coagulant. At appropriate time intervals, 0.5-1.0 ml of blood was removed from each rabbit via marginal ear vein using 22 gauge needles through a butterfly cannula.^{13,14} Blank blood samples were removed from each group before initiation of the treatment. The last blood sample was taken at 4.5 h. After centrifugation at 10,000 rpm for 15 min to separate the plasma, samples were immediately stored frozen at -20°C until analysis.

Quantification of losartan potassium from rabbit plasma

Losartan potassium was measured from plasma samples by LCMS based on a method reported by Jalalizadeh H. et al., using the LCMS/MS API-3000(SCIEX). Diclofenac sodium was used as the Internal Standard (IS) of strength 5 μ g/ml.¹⁵ The extraction method used involved precipitation with acetonitrile as the protein precipitating agent. The frozen samples were thawed and allowed to reach room temperature. Standard solutions for the calibration curve were prepared by spiking pooled rabbit blank plasma with 20 µl of losartan potassium stock solution. To the standard and test samples, 20 µl of the plasma was mixed with 20 µl of the IS and while vortexing 250 µl of acetonitrile was added to precipitate the proteins. The mixture was centrifuged at 4500 rpm for 10 min and 200 µl of supernatant was transferred. 10 µl of the supernatant was injected into the column. The samples were injected into a C-18 column (Chromolith, RP-18e, 100-4.6) and a flow rate of 0.8 ml/min was maintained. The mobile phase utilized was a mixture of acetonitrile: 0.1% Formic acid

(40:60 %v/v) controlled by gradient elution. The drug was detected by a quadrupole mass spectrometer system using positive ion electrospray. In the case of the standard solutions, good linearity was obtained in the range of 2.0-700 ng/ml with a correlation coefficient of 0.998. The limit of detection was 0.5 ng/ml. The drug concentrations that were determined from the test samples were subjected to statistical analysis by one way analysis of variance (ANOVA). Statistical differences were considered significant at P<0.005.

RESULTS AND DISCUSSION

Evaluation of films for physical characteristics

From the results of the tests conducted (Table 2), it is observed that the weight and thickness of all film samples was uniform within each formulation. The drug content in all the formulations was observed to be more or less uniform. The measured surface pH was found to be close to neutral in all the formulations which means that they have less potential to irritate the buccal mucosa and therefore they should be fairly comfortable. Moreover all the films had smooth surfaces without any rough texture. HPMC films are observed to be tougher, smoother, stronger and with better clarity whereas SCMC films tend to be brittle, slightly rough and have poorer clarity.

With the exception of F1, F4 and F5, the folding endurance of all film formulations exceeded 300 indicating that they are tough and flexible. The SCMC films tend to be brittle, hence the poor folding endurance whereas HPMC films are observed to be tougher and more flexible.

Measurement of and Tensile Strength

The tensile testing gives a measure of the strength and elasticity of the film determined by the parameters, Tensile strength (TS) and Elongation at break (E/B). A low TS and E/B is shown by weak and soft polymers while hard and brittle polymers exhibit moderate TS and low E/B; soft and tough polymers are characterized by a moderate TS and high E/B while hard and tough polymers produce high TS and E/B.⁴ Among the film formulations tested, F4 containing SCMC shows the highest TS and moderate E/B. F2 containing HPMC on the other hand shows a moderately high TS and E/B showing that it is sufficiently strong enough for handling and use. These results are shown in (Table 3).

Measurement of Swelling Index, bioadhesive force and *ex vivo* mucoadhesion time

The measurement of Swelling Index indicated that maximum swelling takes place in the formulations con-

taining higher proportions of SCMC and HPMC with faster rate of swelling in the former case. Increase in the content of CP 934 results in slower uptake of water. Since Losartan potassium is a water soluble drug, the water uptake and swelling will also be more. The swelling profile of all the formulations is shown in (Figure 1).

Results of bio-adhesive force measurement (Table 3) show that the force required to detach the film from the mucosal surface depends on the type of polymers used. The bioadhesive strength values of SCMC films were slightly higher than HPMC films for similar compositions. This is probably due to the fact that the incorporation of SCMC in films can increase the surface charge density of the films which could result in stronger bio-adhesion. Moreover the hydration rate was faster for SCMC films which achieved maximum swelling at shorter periods thereby promoting interpenetration of the polymer chain with the mucosa. For the same reason SCMC films also eroded faster than HPMC films. In both groups of films it is observed that increase in Carbopol resulted in significant increase in bio-adhesive force as evident in (Table 3).

Since carbopol is a highly mucoadhesive polymer, it was observed the mucoadhesion time increased as the content of this polymer increased with a maximum of 8.1 h in the case of F1. In spite of the superior mucoadhesive strength of the SCMC films, the measured mucoadhesion time was comparatively less than that of the HPMC films owing to the rapid erosion of the former. These values are displayed in (Table 3).

In vitro drug release studies

In vitro drug release studies in simulated saliva show that almost all formulations exhibit maximum release at 150-180 min or 3 h with the exception of F3 and F6 which show maximum release at 240 min or 4 h. The drug release profiles for all formulations are given in (Figure 2). The range of release was 94.34% (F2) to 98.97% (F5). The use of higher percentage of carbopol in the films hastened the release of the drug. This may be due to the ionization of CP at pH 6.6, a pH environment higher than its ionization constant (pK) of 6. Ionization of CP leads to the development of negative charges along the backbone of the polymer. Repulsion of like charges uncoils the polymer into an extended structure, leading to slightly higher uptake of water that might have contributed to higher drug release from the polymer matrix systems.¹⁶ Moreover, losartan potassium is quite water soluble and therefore increases the hygroscopic nature of these films. The drug release was faster from SCMC films than HPMC films since the ionic nature of SCMC causes hydration and swelling of the polymer at a faster rate.

Table 1: Composition of drug loaded polymeric films						
Ingredients in % w/v	F1	F2	F3	F4	F5	F6
HPMC	0.3	0.5	0.7			
SCMC				0.3	0.5	0.7
CP 934	0.7	0.5	0.3	0.7	0.5	0.3
PEG 400	1.0	1.0	1.0	1.0	1.0	1.0
Losartan potassium	0.55	0.55	0.55	0.55	0.55	0.55

Table 2: Physical characteristics of the formulated drug loaded films						
Formula Code	Weight (mg)	Thickness (mm)	Surface pH	Folding Endurance	Content uniformity (mg)	
F1	48.1 ± 3.24	0.67 ± 0.05	6.35 ± 0.14	260 ± 23	9.32 ± 0.12	
F2	46.1 ± 3.54	0.49 ± 0.02	7.90 ± 0.15	>300	9.60 ± 0.23	
F3	48.0 ± 2.10	0.31 ± 0.02	6.15 ± 0.21	>300	9.54 ± 0.35	
F4	32.7 ± 0.42	0.42 ± 0.01	6.25 ± 0.13	215 ± 10	9.72 ± 0.28	
F5	23.5 ± 2.54	0.38 ± 0.08	6.08 ± 0.15	237 ± 18	9.43 ± 0.15	
F6	32.3 ± 3.15	0.22 ± 0.06	6.95 ± 0.15	>300	9.55 ± 0.22	

All observations represent the mean ± S.D and n=10 for weight and thickness and n=3 for others.

Table 3: Results for evaluation of mechanical properties of formulations					
Formula Code	Bioadhesive Force (kg/m/s²)	Tensile strength (kg/mm²)	% Elongation	Mucoadhesion time (min)	
F1	19.21 ± 1.02	1.667 ± 0.24	20.00 ± 1.32	490 ± 3	
F2	16.14 ± 0.63	1.760 ±0.36	37.77 ± 3.66	365 ± 3	
F3	15.03 ± 1.22	0.930 ± 0.71	74.43 ± 4.20	300 ± 6	
F4	19.76 ± 0.46	2.233 ± 0.32	30.00 ± 1.84	325 ± 3	
F5	16.74 ± 1.42	1.270 ± 0.08	18.75 ± 6.75	298 ± 2	
F6	15.59 ± 0.34	1.388 ± 0.61	15.43 ± 2.57	214 ± 4	

All observations re	present the mean	± S.D and n=3
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Table 4: Results for kinetic analysis of <i>in vitro</i> drug release data								
Release Model		Formulation Code						
		F1	F2	F3	F4	F5	F6	
Zero Order	R²	0.7028	0.7535	0.7605	0.7425	0.8980	0.8791	
	k	0.7218	0.8433	0.5306	0.8715	0.7154	0.4857	
First Order	R²	0.9807	0.9579	0.9853	0.9660	0.9947	0.9702	
	k	0.0145	0.0216	0.0146	0.0019	0.0104	0.0016	
Higuchi	R²	0.9358	0.9711	0.9661	0.9244	0.9515	0.9966	
Matrix	k	8.6067	9.0427	7.2013	9.5548	8.4938	6.4241	
Peppas	R²	0.9553	0.9788	0.9792	0.9875	0.9853	0.9947	
	k	10.883	9.0059	8.5793	12.2913	12.1931	7.1148	
	n	0.5624	0.5105	0.4916	0.5727	0.4948	0.5807	
Best fit Mode	el	First Order	Peppas	First Order	Peppas	First Order	Matrix	

Table 5: Pharmacokinetic parameters of losartan potassium administered in rabbits					
Formulation	Pharmacokinetic parameters*				
Formulation	C _{max} (ng/ml)	T _{max} (h)	AUC _{0-∞} (ng-h/ml)		
Oral solution of Losartan potassium (10 mg/5 ml)	102.25 ± 1.434	1.0 ± 0.33	257.618 ± 3.566		
F1	183.22 ± 3.049	1.5 ± 0.32	509.43 ± 4.427		
F4	191.06 ± 2.215	1.5 ± 0.26	528.72 ± 1.628		

*All values are represented as mean \pm S.D and n=4.

Kinetic analysis of in vitro release data

In order to determine the release mechanism that provides the best description to the pattern of drug release, the *in vitro* release data were fitted to zero-order, first-order, and Higuchi matrix model. The release data were also kinetically analyzed using the Korsmeyer–Peppas model and the release exponent (n) describing the mechanism of drug release from the matrices was calculated by regression analysis using the following equation.¹⁷

$Mt/M\infty = Kt^n$

Where $Mt/M\infty$ is the fraction of drug released (using values of M/M^{∞} within the range 0.10–0.60) at time t and K is a constant incorporating the structural and geometric characteristics of the release device. When n=0. 5, Case I or Fickian diffusion is indicated, 0.5 <n < 1 for anomalous (non-Fickian) diffusion, n=1 for Case II transport (Zero order release) and n > 1 indicates Super case II transport. From the mathematical treatment of the in vitro release data of losartan potassium from buccal films, the values of K, n and R² (coefficient of determination) has been obtained as presented in (Table 4). The kinetics of drug release from all formulations appeared to follow first order as seen from the regression values. For the mechanism of release, the values of n obtained by the linear regression of log $(Mt/M\infty)$ vs. log t, were between 0.5 to 1 for formulations F1, F2, F4 and F6 indicating non-fickian diffusion as the release mechanism, and less than 0.5 in the case of F3 and F5. The best fit model with the highest correlation r and determination R² coefficients for F1, F3 and F5 was the First order equation, where as for F2 and F4 it was the Korsmeyer and Peppas model and F6 followed the Higuchi matrix model.

In vivo buccal permeation studies of drugs from mucoadhesive films in rabbits

Based on *in vitro* release data as well as mucoadhesion properties, the films selected for *in vivo* studies were F1 and F4. The following treatment groups were used:

Group I- Oral solution of losartan potassium (10 mg/2 ml)

Group II- Formulation F1

Group III- Formulation F4

During the study it was observed that all films remained intact and adhered well to the buccal mucosa of the rabbit. There were also no noticeable signs of any irritation or redness at the sites of application.

The calibration curve of losartan potassium from spiked rabbit plasma displayed excellent linearity over



Figure 1: Swelling profile of film formulations in simulated salivary fluid



Figure 2: *In vitro* release profile of losartan potassium from formulations in simulated saliva



Figure 3: Calibration Curve of losartan potassium in rabbit plasma

the concentration range investigated ($R^2=0.998$) as displayed in (Figure 3).

The mean plasma concentration-time profiles of losartan at different time intervals following the application of buccal patches and oral administration of the solution in each group of rabbits is shown in (Figure 4). Pharmacokinetic parameters such as C_{max} , T_{max} and $AUC_0^{-\infty}$ were determined using model-independent methods with non-



Figure 4: Mean Plasma drug concentration-time profile of Losartan potassium from buccal films and oral solution



Figure 5: Plot of Percentage Drug absorbed from F1 and F4 with time after de-convolution of plasma level data in rabbits



Figure 6: IVIVC of losartan potassium from F1

linear least-squares regression analysis (WinNonlin, Pharsight) from the plasma drug concentration-time profiles of each individual rabbit. C_{max} is the peak plasma drug concentration which varies with the dose administered or route of administration, T_{max} is the time required to reach peak plasma drug concentration and is independent of the dose, and AUC (Area under the curve) were calculated. AUC was determined; using the trapezoidal rule. The average values of these pharmacokinetic parameters were determined and displayed in (Table 5).



Figure 7: IVIVC of losartan potassium from F4

Statistical Analysis of data from rabbit plasma drug concentrations

When the mean plasma drug concentration data from the different treatment groups were subjected to statistical analysis by one way ANOVA, it was found that differences between the groups I that received the oral solution and II or III which received the patches containing the same dose of the drug, were statistically significant with respect to $\mathrm{C}_{\mathrm{max}},\,\mathrm{T}_{\mathrm{max}}$ and AUC. From the pharmacokinetic parameters determined, it is clear that the calculated $AUC_{0-\infty}$ was found to be significantly higher (P<0.005) from the formulations than from the oral solution containing the same amount of drug. It is known from published literature that losartan potassium suffers from poor oral bioavailability due to hepatic first pass metabolism. Thus, this study confirms the fact that buccal delivery of losartan potassium can increase its bioavailability and thereby improve its therapeutic efficacy.

The T_{max} values are greater (P<0.005) in the case of the formulations as compared to that of the oral solution, indicating the slower release of the drug from buccal films thereby providing prolonged effects For the same reason the C_{max} values of F1 and F4 are also significantly greater (P<0.005) than that of the oral solution in spite of the same dose which is indicative of the reduced bioavailability of the drug when given orally. All three parameters however, did not show significant difference between F1 and F4 (P>0.05).

In vitro-in vivo correlation

Since losartan potassium is a Class I drug as per BCS classification, for immediate release formulations, IVIVC correlation is not required or expected but biowavers are not applicable to buccal dosage forms. Moreover, incorporating this drug in a sustained release film will place it in Class II (low solubility, high permeability). Hence Level A correlation was undertaken for

the formulations where *in vivo* percentage drug absorbed was plotted against *in vitro* percentage drug released to determine the correlation coefficient.^{18,19}

The percentage drug absorbed was determined using deconvolution methods such as the Wagner Nelson method using the following equation.^{20,21}

Fa =
$$[(C_t + k_e AUC_{0-t}) / k_e AUC_{0-\infty}] \times 100$$

Where Fa is the fraction of drug absorbed, C_t is the plasma drug concentration at time t, k_e is the overall elimination rate constant, AUC_{0-t} and AUC_0-_{∞} are areas under the curve between time zero and time t and between time zero and infinity, respectively. The results of percentage drug absorbed *in vivo* through the buccal mucosa were correlated with *in vitro* percentage drug released for the same time intervals as shown in (Figure 5). Good *in vitro–in vivo* correlation was obtained for the two formulations as seen in the correlation graphs in (Figure 6 and 7).

CONCLUSION

The results of the evaluation studies reveal that a combination of mucoadhesive polymers in the right proportions were required to produce films with satisfactory adhesion properties as well as to be able to prolong the release of the drug. Carbopol was imperative in imparting mucoadhesive property to the films along with the presence of SCMC or HPMC. Further, the *in vivo* studies have not only confirmed the ability of the formulations to prolong drug release but also proved that it is possible to improve the bioavailability of the drug by buccal administration. The results of drug absorption studies in rabbits can be easily extrapolated to human beings. In terms of drug release and satisfactory physical properties including mucoadhesion, F1 and F4 can be considered as promising formulations for clinical application.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

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PICTORIAL ABSTRACT



ABBREVIATIONS USED

HPMC: Hydroxy propylmethyl cellulose; SCMC: Sodium carboxymethyl cellulose; CP: Carbopol; PEG 400: Polyethylene Glycol 400; S.I: Swelling Index; TS: Tensile Strength; LCMS: Liquid Chromatography-Mass Spectrometry; IS: Internal Standard; BCS: Biopharmaceutics Classification System; ANOVA: Analysis of Variance; IVIVC: *In vitro in vivo* correlation.

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SUMMARY

- Mucoadhesive films of losartan potassium were prepared from hydrophilic polymers, hydroxy propylmethyl cellulose/sodium carboxymethyl cellulose and carbopol 934 P with suitable plasticizers by solvent casting.
- The films were characterized for physicochemical properties as well as for *in vitro* release and *in vivo* buccal absorption in rabbits.
- *In vitro* release profiles exhibited sustained delivery of the drug over 4 hours and drug release kinetics was first order.
- Pharmacokinetic studies in rabbits that the values of $C_{max'} T_{max}$ and AUC in the groups that received the optimized formulations were statistically different (P<0.005) from that of the groups that received the oral solution at the same dosage level.