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The effect of conjugation on different polymers in bioadhesive films of losartan

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ABSTRACT

The objective of the present study was to develop mucoadhesive buccal films of losartan potassium using sodium alginate, chitosan, and their conjugated derivatives at different concentrations by solvent casting technique. Glycerin was used as plasticizer, at different weight ratios. The conjugation of polymers (sodium alginate-cysteine and chitosan-thioglycolic acid) was confirmed by FTIR study. The resulted conjugated sodium alginate and chitosan displayed 475.64±24.31 and 305±31.21 µmol thiol groups per g conjugate, respectively. The developed films were evaluated by different parameters such as weight uniformity, thickness, surface pH, swelling index, *in vitro* residence time, mucoadhesive strength, *in vitro* and *ex vivo* drug permeation. The results indicated that the formulations S5, C4, Cs and Cc showed better characteristic properties and drug permeation. Good results were obtained both *in vitro* and *ex vivo* conditions for optimized film. A higher glycerin percent in films increased the drug permeation, particularly at a polymer concentration of 10 to 30% w/v (S2-S4 and C2-C4). A further increase of glycerin (40 to 50% w/v) did not improve losartan potassium permeation (S6 and C6). Formulations Cs and Cc containing conjugated sodium alginate and chitosan resulted in a significantly higher bioadhesion, swelling with better drug permeation over long period than films comprising unmodified polymers. Stability studies on optimized formulations indicated that the formulations are stable as no significant difference was observed in either drug content or drug release.

Keywords: Buccal drug delivery, Sodium alginate–cysteine conjugate, Chitosan-thioglycolic acid conjugate, Losartan potassium, Permeation study, Sustained release.

INTRODUCTION

Within the last decade, the concept of mucoadhesive polymeric excipients has gained a new dimension by introducing thiolated polymers at the pharmaceutical arena. Thiolated polymers or designated thiomers are new generation mucoadhesive basis polymers, which display thiol bearing side chains. Thiomers, represent a new class of efficient mucoadhesive polymers with improved mucoadhesive and permeation enhancing properties.

In this study thiolated chitosan and thiolated sodium alginate was chosen as a polymeric drug carrier. Only a few studies have so far been performed on the usefulness of thiolated polymers as drug delivery systems and no buccal applications has been investigated for these films yet. Few researchers have tried to deliver a drug systemically by using thiolated polymers through the oral cavity including peptide drug delivery [1], microparticles [2]. In the present study, Losartan potassium was chosen as the model drug. Losartan potassium is an orally active angiotensin II receptor antagonist indicated for the treatment of hypertension. [3] The conventional oral dosage form of Losartan potassium undergoes

substantial first pass metabolism and the systemic bioavailability is only about 33%. Peak plasma concentrations are achieved in 1 h. The antihypertensive effect, measured at trough using once daily dosing, is inadequate. A twice daily regimen at the same daily dose, may give a more satisfactory response. Twice daily regimen, however, leads to patient incompliance, fluctuations in the drug blood level and other adverse effects associated with the conventional drug delivery system. [4] All these properties, such as extensive hepatic first pass metabolism (67%), short biological half-life (2 h), low dose (25– 50 mg), low oral bioavailability (33%)

MATERIALS AND METHODS

All the materials obtained and used are of analytical grade. Losartan potassium obtained from Karnataka antibiotics Pvt Ltd., Bangalore. Sodium alginate and Chitosan obtained from Sigma-Aldrich chemicals Pvt Ltd., Mumbai. Carbodiimide hydrochloride and Thiogycolic acid obtained from Spectrochem Pvt Ltd., Mumbai. L-Cysteine Loba obtained from Chemicals Pvt Ltd., Mumbai. Glycerin obtained from S.D. Fine Chem. Pvt Ltd., Mumbai. Dialysis membrane 110 obtained from Himedia private Pvt Ltd., Mumbai

Methodology

Preparation of phosphate buffer pH 6.8

6.8 g of potassium dihydrogen phosphate was dissolved in 250 ml of distilled water and this solution was diluted to 1000 ml in a volumetric flask. To it 1.2 gm of NaOH in 112 ml distilled water was added. Then the volume was made up to 1000 ml with distilled water.

Calibration curve of losartan potassium in phosphate buffer pH 6.8

Calibration curve of losartan potassium was developed by suitably diluting the previously prepared stock solution (1000 µg/ml) of drug using phosphate buffer pH 6.8. Absorbance of serially diluted (1-10 µg/ml) solutions were measured using a UV-spectrophotometer (UV-1601, SHIMADZU, Japan) at 227 nm against a suitable blank. A calibration curve was plotted using Microsoft Excel software.

Synthesis of conjugated polymers

Conjugation of polymer chitosan - thioglycolic acid [5, 6]

500 mg of chitosan was hydrated in 4 ml of 1 M HCl and dissolved by the addition of demineralized water in order to obtain a 1 % w/v solution of chitosan hydrochloride. Thereafter, EDAC was added in a final concentration of 50 mM. After EDAC was completely dissolved in the chitosan hydrochloride solution, 500 mg of TGA was added. The pH was adjusted to 4.0 using 1 M NaOH. The reaction mixture was incubated for 3 h at room temperature. The resulting chitosan – thioglycolic acid conjugate was isolated by dialyzing at 10 ºC against 1mM HCl, followed by two times against the same medium but also containing 1% NaCl and then exhaustively against 1 mM HCl. Thereafter, sample was stored in a desiccator under vacuum until completely dried. The dried conjugated chitosan was powdered using glass mortar-pastel and passed through sieve number 60 and stored in desiccator until further use.

Conjugation of polymer sodium alginate-L-cysteine [7, 8]

1 gm of sodium alginate was dissolved in 100ml demineralized water in order to obtain a 1 % w/v polymer solution. Thereafter, EDAC was added in a final concentration of 50 mM. After EDAC was completely dissolved in the chitosan hydrochloride solution, 500 mg of L- cysteine monohydrate hydrochloride was added and the pH was adjusted to 4.0 with 1 M HCl. The reaction mixture was incubated for 2 h under stirring at room temperature. The pH was adjusted to 6.0 using 1 M NaOH. The reaction mixture was incubated for 3 h at room temperature under permanent stirring. The resulting alginate–cysteine conjugate was isolated by dialyzing at 10 ºC against 1mM HCl, followed by two times against the same medium but also containing 1% NaCl and then exhaustively against 1 mM HCl. Thereafter, sample was dried in desiccator under vacuum. The dried conjugated chitosan was powdered and stored in desiccator until further use.

Confirmation of prepared polymer conjugates

FT-IR Spectrophotometric analysis

The sample of drug and drug-polymer mixture were subjected to attenuated total refraction and scanned from 4000 cm^{-1} to 500 cm^{-1} using FT-IR spectrophotometer (Bruker-α-T, Germany)

Determination of charring point

The charring point of the polymer conjugates were determined by taking approximately 5 mg of the sample in a glass capillary tube sealed at one end. The sample-containing capillary was placed in melting point apparatus and the temperature was increased gradually. The temperature at which sample charred completely was noted down as the charring point of that sample.

Determination of the thiol group content

The thiol concentration on polymer conjugates and controls were determined by iodometric titration. [9] Each polymer (3.0 mg) was hydrated in 1.0 ml of demineralized water. The pH was adjusted to between 2 and 3 by adding 1 M HCl. After the addition of 200µl of aqueous starch solution (1%; m/ v), samples were titrated with an aqueous iodine solution 1.00 mM until a permanent light blue colour maintained.

Preparation of plain and conjugated mucoadhesive buccal films

Buccal patches were prepared using solvent casting technique [10, 11] using plain and conjugated polymers. Different concentrations of polymers and plasticizer glycerin were used in preparation of films; the films of respective compositions were fabricated using polymers along with drug and solvent. The compositions of the formulated films were given in the Table 1. Initially, films were prepared using only plain chitosan and sodium alginate. The polymers were weighed accurately, added to 30 ml of acetic acid (1% v/v) and distilled water respectively, and soaked for overnight and triturated until to form a homogenous system then glycerin was added. The drug was added to the above gel and triturated to form a homogenous gel. In order to avoid entrapment of the air bubbles inside the film, the entire gel was subjected to sonication. Then these gels were cast into poly ethylene coated glass petridish and allowed to dry in a hot air oven with levelled surface maintained at 40 °C till a flexible film was formed (48 h). The dried films were cut into patches of $1cm^2$ and packed in aluminium foil then stored in airtight desiccator prior to use. These films were examined in order to select the film having the best characteristics to prepare conjugated buccal films.

EVALUATION OF THE PREPARED FILMS

Content uniformity

To ensure uniform distribution of losartan potassium in film, a content uniformity test was performed. The film of $1cm^2$ was added to 50 ml of Phosphate buffer (pH 6.8) contained in a 100 ml volumetric flask was placed on mechanical shaker and continuously shaked for 24 h. Then the solution was filtered and the filtrate was examined for the drug content by using UV-spectrophotometer (UV-1601, SHIMADZU, Japan) at 227 nm. The experiments were carried out in triplicate for the films of all formulations and average values were taken as final reading.

Film thickness

The diverging thickness of the films can drastically alter the drug content. Uniform thickness is highly important for the films composed of potent drugs. The thickness of film was measured at different positions with the help of Dail micrometer (Mitutoyo, Japan) with the smallest possible unit measurement count of 0.1 mm. The measurement was done for about three films of each formulation and average values were taken.

Weight variation

The formulated films were evaluated for their uniformity in weights. Three films of every formulation were randomly taken and weighed individually on a digital balance. Average weight of the films was noted as weight of the films.

Surface pH

Buccal patches were left to swell for 2 h on the surface of an agar plate. The surface pH was measured by means of a pH paper placed on the surface of the swollen patch. A mean of three readings was recorded.

Swelling studies [12]

Three patches were tested for each formulation. The films of $1cm^2$ were weighted individually (W_o) and placed separately in Petri dishes containing 5 ml of phosphate buffer (pH 6.8) solution. At regular intervals (1, 2, 3, 4, 5, 6, 7 and 8 h), the films were removed from the Petri dishes and excess surface water was removed care fully using the filter paper. The swollen films were then reweighed (W_t) and

swelling index (%S) was calculated using the following formula:

$$\%S = \frac{W_t - W_o}{W_o}$$

Folding endurance

A modified USP tablet disintegrating tester was used to determinine the folding endurance of the membrane. [13] It consisted of fixed and movable jaws that could be moved up and down at the rate of 30 strokes per minute. The distance between the 2 jaws at their farthest and closest were 6 centimeter and 0.5 centimeter respectively. The membrane (6 cm length) was clamped between the jaws in such a way that the jaws were at their closest, the membrane stretch across its middle and when at their farthest, the membrane was in a stretched condition. Thus for every stroke of the movable jaw the membrane went through one cycle of bending and stretching. The folding endurance is expressed as the number of strokes required to either break or develop visible cracks on the membrane. The test was conducted for 20 min equating 600 strokes. The examination was done for about three films of each formulation and average values were taken.

Bioadhesion force

The tensile strength required to detach the bioadhesive film from the mucosal surface was applied as a measure of the bioadhesive performance. The apparatus was locally assembled and was a modification of the apparatus previously described by Parodi *et al*. [14] The device was mainly composed of a two-arm balance. The left arm of the balance was replaced by a small platinum lamina vertically suspended through a wire. At the same side, a movable platform was maintained in the bottom in order to fix the model mucosal membrane. For determination of the bioadhesion force, the mucoadhesive film was fixed to the platinum lamina using cyanoacrylate adhesive. A piece of goat buccal mucosa, 3 cm long, was also glued to the platform. The exposed film surface was moistened with 30 µl of phosphate buffer (pH 6.8) and left for 30 seconds for initial hydration and swelling. The platform was then raised upward until the hydrated film was brought into contact with the mucosal surface. A preload of 20 g was placed over the platinum lamina for 3 min as initial pressure. On the right pan, a constant weight of 5 g was added at 2 min intervals. The total weight required for complete detachment of the film was recorded and the bioadhesion force was calculated per unit area of the film as follows

$$F = \frac{(W_w \times g)}{A}$$

Where "F" is the bioadhesion force ($kgm^{-1}s^{-2}$), "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 film (cm^2). The adhesion force data reported represent the mean of three determinations.

Residence time

The *in vitro* residence time was determined using a locally modified USP disintegration apparatus. [15] The disintegration medium was composed of 500 phosphate buffer (pH 6.8) maintained at 37 °C. A segment of goat buccal mucosa, 3 cm long, was glued to the surface of a glass slab, vertically attached to the apparatus. The mucoadhesive film was hydrated from one surface using 15 µl Phosphate buffer (pH 6.8) and then the hydrated surface were brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down so that the film was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time necessary for complete erosion or detachment of the film from the mucosal surface was recorded (mean of triplicate determinations).

In vitro permeation studies

The modified Franz diffusion cell was used to permeation studies, it consists of two compartments, one is donar compartment and another is receptor compartment of 18 ml capacity and having 0.785 cm^2 effective surface area. The receptor compartment was covered with jacket to maintain temperature 37 ºC. The study was carried out on formulations using dialysis membrane. [16] The dialysis membrane was previously soaked for 12 h in the phosphate buffer (pH 6.8). Then the film formulations of 1cm^2 were mounted between two chambers and in receptor chamber phosphate buffer pH 6.8 was filled and tied firmly with the help of rubber band to the specially designed diffusion cell. The whole assembly was placed on a magnetic stirrer, and the solution in the receptor compartment stirred with the help of a teflon coated bead at a constant speed and the temperature of

whole assembly was maintained at 37 ± 0.5 ºC by circulating hot water inside the water jacket. The release study was carried out for 8 h. The samples (5 ml) were withdrawn and filtered at predetermined intervals up to 8 h and replaced with Phosphate buffer (pH 6.8) to maintain the sink conditions. The drug content was analyzed with the help of UV-spectrophotometer (UV-1601, SHIMADZU, Japan) at 227 nm. The experiment was repeated in triplicate for all formulations.

Ex-vivo permeation studies

From the local slaughterhouse the buccal mucosa was collected and immediately transported to the laboratory in Ringer's solution. [17] The buccal mucosa, with a part of submucosa, was carefully separated from fat and muscle using scalpel. Then buccal epithelium was isolated from the underlying tissue and stabilized in phosphate buffer (pH 6.8). The buccal epithelium was used with in 2 h upon removal. The study was carried out on formulations using buccal epithelium. This experiment was carried out based on same procedure give in section.

Release Kinetics

In vitro release data were fit to following kinetic equation to determine the order and mechanism of drug release. [18, 19]

Zero order kinetics : $Q_t = K_o t$

First order kinetics : $Q_t = Q_o e^{-Kt}$

Higuchi's square root model : $Q_t = K_H \sqrt{t}$

Where, Q_t is amount of drug released at a time *t*, Q_o is the initial amount of drug in the dissolution medium. *K*, K_o and K_H are release constants.

In order to further determine the mechanism of drug release, data was fit to Korsemeyer-Peppas empirical power law equation. [20]

$M_t/M_\infty = Kt^n$

Where M_t/M_∞ is the fraction of drug released at time'*t'*, *K* is the structural and geometrical constant and '*n*' is the release exponent.

When n = 0.5, fickian diffusion is observed and the release rate is dependent on t, while in other conditions $0.5 < n < 1$ is consider to release drug by a combination of drug diffusion as well as polymeric chain relaxation (anomalous or non fickian transport). When *n*=1, the drug release is considered to show linearity towards zero order release kinetics.

Stability studies and ageing

Plain and drug loaded patches were packaged in aluminium foil and stored in glass bottles closed with screw caps. These bottles were subjected to accelerated stability testing using stability chamber (Remi instruments Ltd., Mumbai) maintained at 37º C and 75 ± 5% RH for 1 month. Fresh and 1 month aged medicated patches were evaluated.

RESULTS & DISCUSSION

Table 1: Composition of losartan potassium mucoadhesive buccal films

Formulation	S1	S2	S3	S4	S5	S6	C1	C2	C3	C4	C5	C6	Cs	Cc
Losartan potassium (mg)	70	70	70	70	70	70	70	70	70	70	70	70	70	70
Sodium alginate (mg)	450	600	600	600	600	600	0	0	0	0	0	0	0	0
Chitosan (mg)	0	0	0	0	0	0	450	600	600	600	600	600	0	0
Glycerin w/v	10%	10%	20%	30%	40%	50%	10%	10%	20%	30%	40%	50%	30%	30%
Conjugated sodium alginate (mg)	0	0	0	0	0	0	0	0	0	0	0	0	600	0
Conjugated chitosan (mg)	0	0	0	0	0	0	0	0	0	0	0	0	0	600
Distilled water (ml)	30	30	30	30	30	30	0	0	0	0	0	0	30	30
1% Acetic Acid (ml)	0	0	0	0	0	0	30	30	30	30	30	30	0	0

Calibration curve of losartan potassium

In this study, mucoadhesive buccal films of losartan potassium were developed using polymers like sodium alginate, chitosan, conjugated sodium alginate and conjugated chitosan at different concentrations. Glycerin was used as plasticizer, at different weight ratios. The mucoadhesive buccal films were prepared by solvent casting technique.

The calibration curve of losartan potassium was developed in the range of 1 to 10 µg/ml at 227 nm. Good linearity with a regression coefficient of 0.999 (r^2 value) was observed. The tested concentration range obeyed Beer's law (Figure 1).

Table 2: Concentration and absorbance obtained for calibration curve of losartan potassium.

Sl No	Concentration (µg/ml)	Absorbance (at 227 nm)
1	0	0
2	1	0.104±0.004
3	2	0.190±0.006
4	3	0.257±0.011
5	4	0.341±0.003
6	5	0.435±0.001
7	6	0.505±0.015
8	7	0.587±0.001
9	8	0.682±0.002
10	9	0.757±0.007
11	10	0.832±0.004

Values are mean±SD, n=3

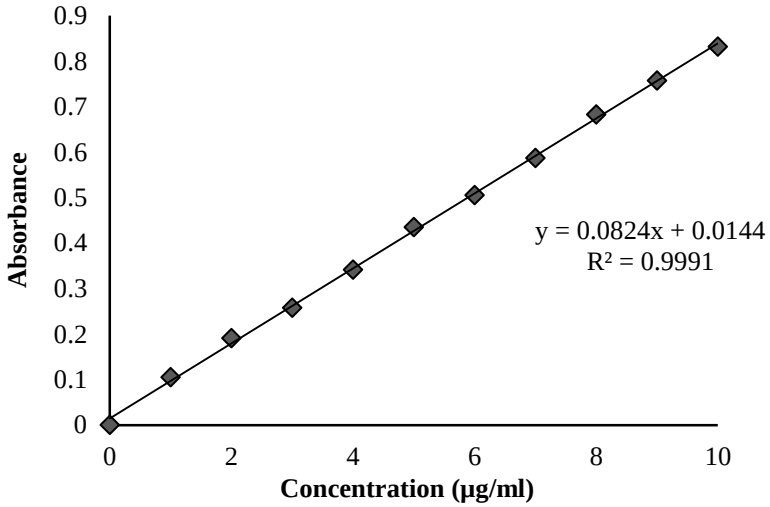

Figure 1: Calibration curve of losartan potassium

CHARACTERISATION OF POLYMER CONJUGATES

FT-IR study

The FTIR spectra of sodium alginate and thiolated sodium alginate are shown in Figure 2 and 3, FTIR spectrum of sodium alginate is attributed to its saccharide structure. In addition, the bands at 1602 cm^{-1} and 1406 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of carboxylate salt groups. In the spectra of conjugated sodium alginate the peak at 1581.27 cm^{-1} was caused by carbonyl (C=O) stretching of the amide and the absorption band at 2577.88 cm^{-1} corresponded to the thiol group (S-H)

stretching which were compared with the IR spectra of the pure sodium alginate polymer and found that these characteristic peaks were absent in pure sodium alginate polymer. [21, 22] It was observed from the spectra of the conjugated sodium alginate that the band represented thiol group (S-H) stretching (at 2577.88 cm^{-1}) confirmed the conjugation of sodium alginate with cysteine and the peak caused by carbonyl (C=O) stretching of amide bond (at 1527.67 cm^{-1}) which has confirmed that the conjugation has occurred through the amide linkage only. The FTIR spectra of conjugated chitosan, showed the bands representing the -C=O stretching of amide bond (at 1645.45 cm^{-1}) and -SH stretching (at 2664.24 cm^{-1}) which were absent in the FTIR spectra of the chitosan. [23] The additional peaks in the FTIR spectra of the conjugated chitosan have confirmed the conjugation of chitosan with TGA. The FT-IR spectra of chitosan and conjugated chitosan are shown in figure 4 and 5.

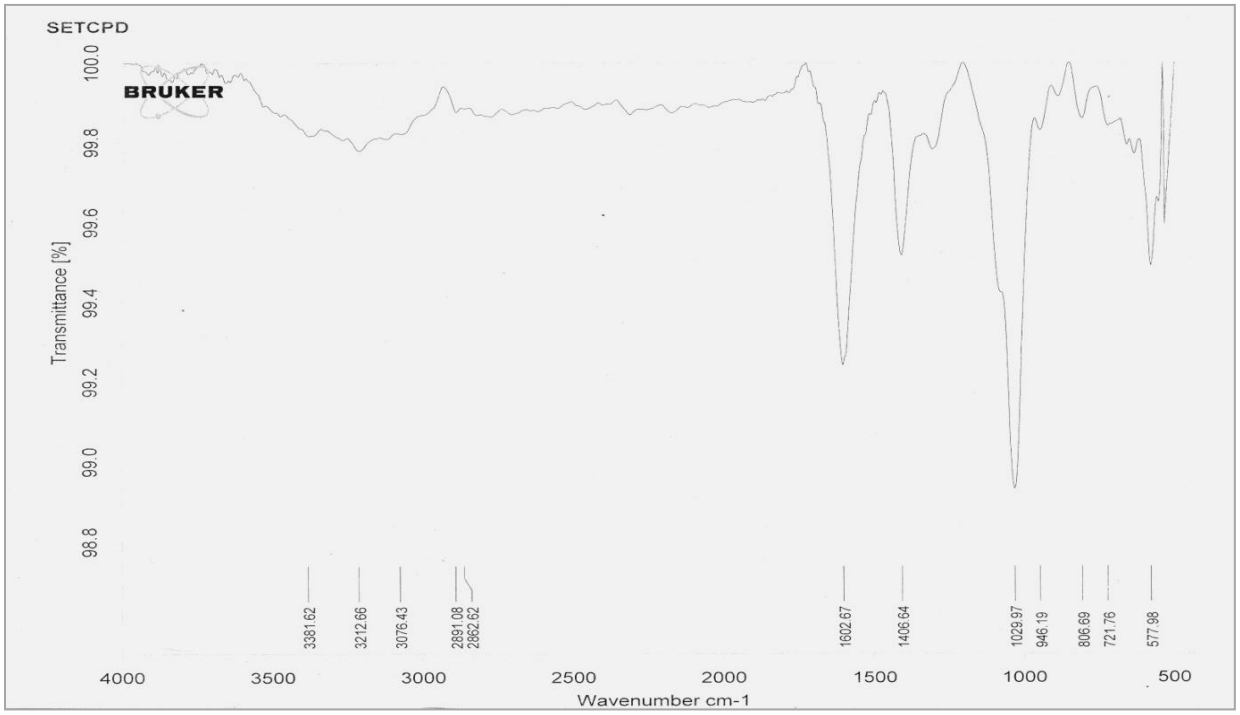

Figure 2: FT-IR spectrum of pure sodium alginate polymer

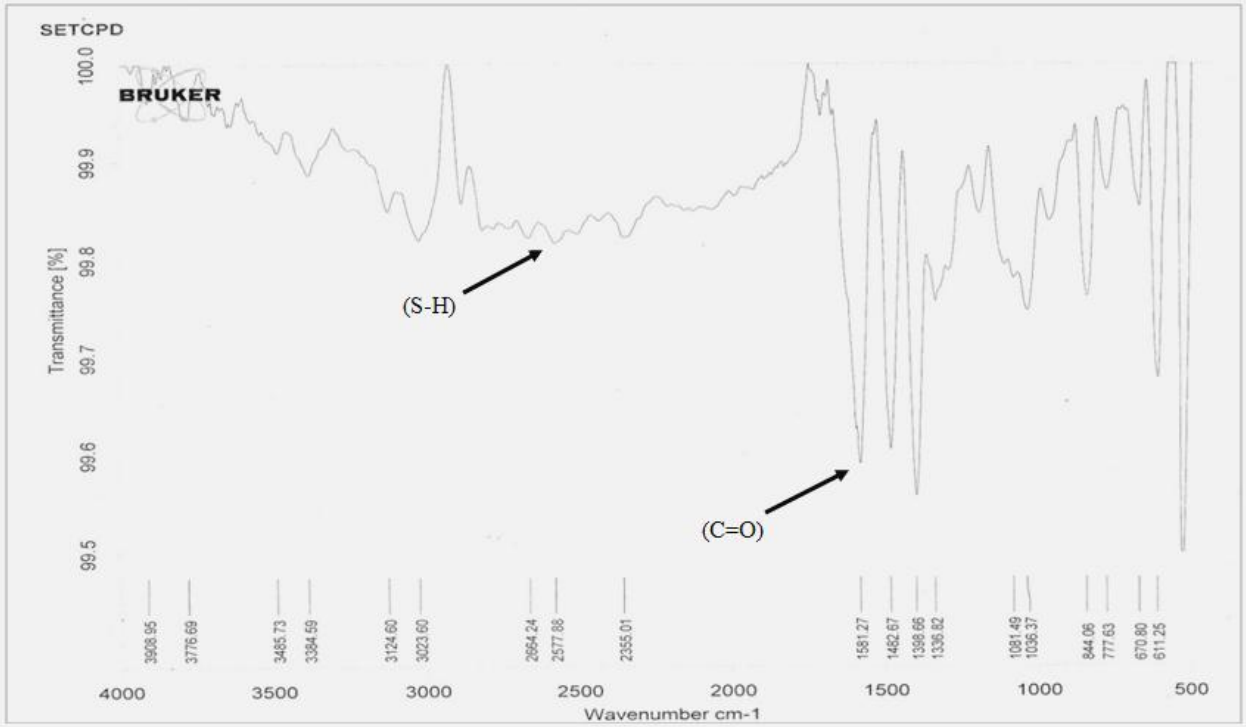

Figure 3: FT-IR spectrum of conjugated sodium alginate polymer

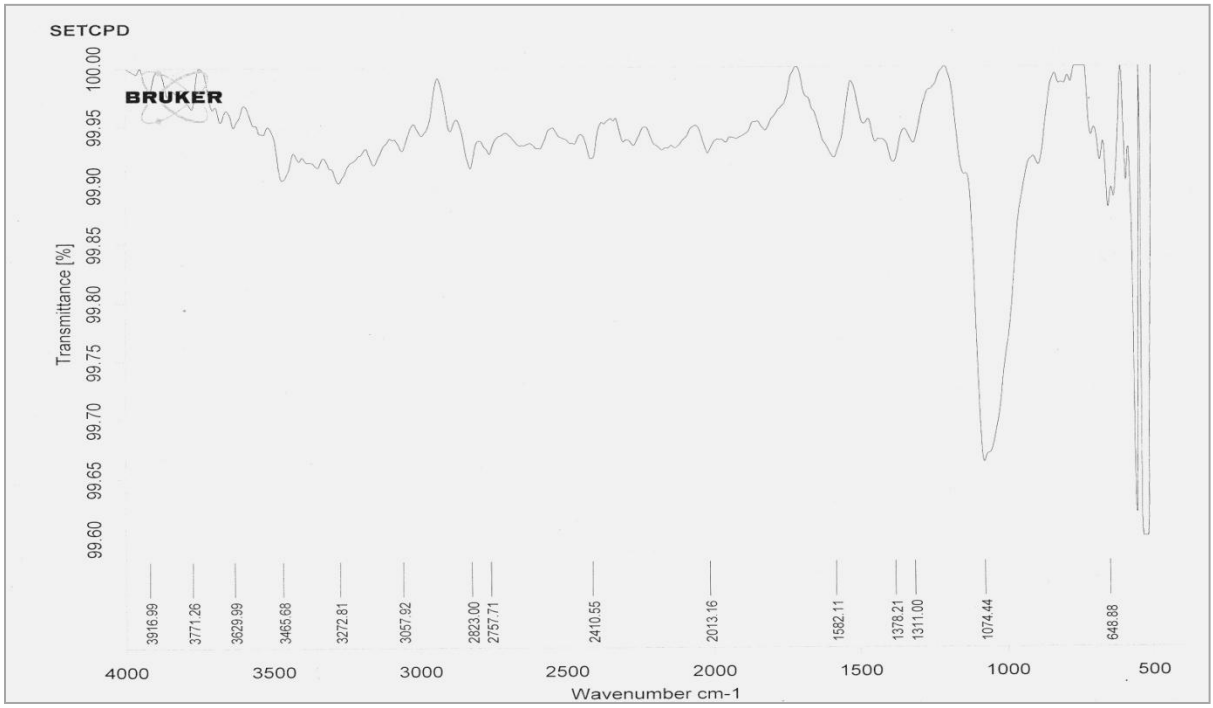

Figure 4: FT-IR spectrum of pure chitosan polymer

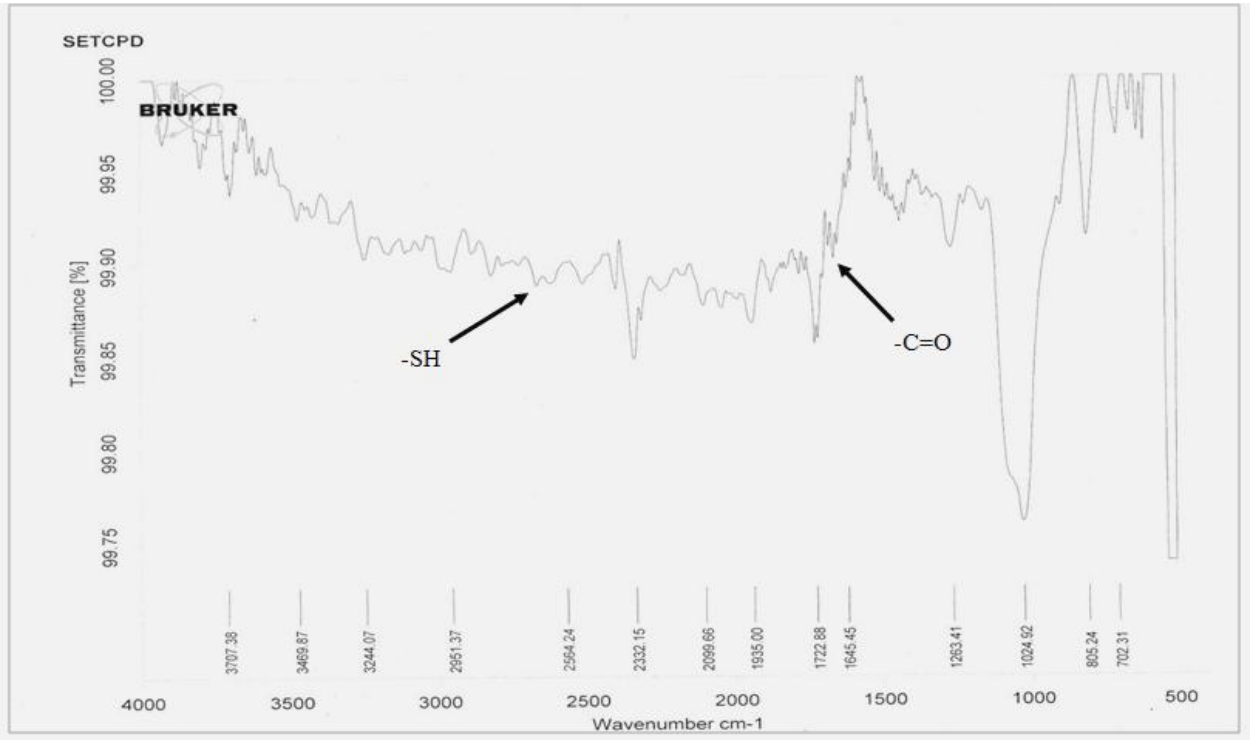

Figure 5: FT-IR spectrum of conjugated chitosan polymer

Determination of the thiol group content

The degree of modification by the covalent attachment of cysteine to alginate and thioglycolic acid to chitosan was quantified by iodometric titration, to determine free thiol groups and conformation of polymer conjugates. The alginate–cysteine conjugate displayed 475.64±24.31 µmol thiol groups per g polymer. The chitosan thoglycolicacid conjugate displayed 305±31.21 µmol thiol group per g polymer. The presence of thiol groups in both polymers confirmed sodium alginate-cysteine and chitosan-thioglycolic acid conjugation (Figure 6).

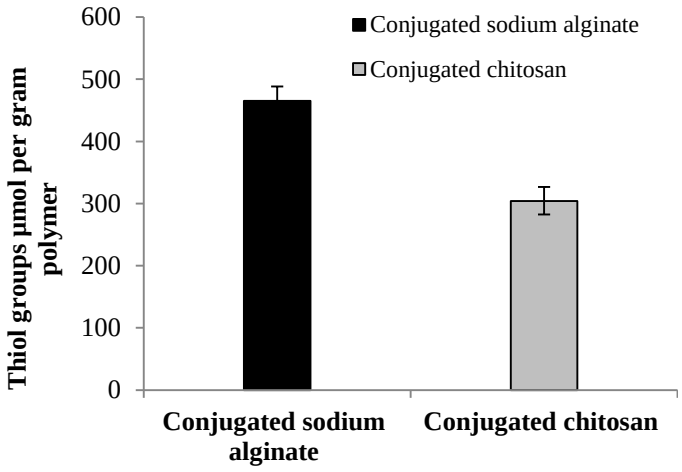

Figure 6: Resulted thiol group content of conjugated polymers

Characteristics of developed formulations

Physical characteristics of the formulated losartan potassium mucoadhesive buccal films.

Table 3 summarizes the physical characteristics of the formulated films. All the formulations exhibited fairly uniform drug content and ranged from 95.29 ± 0.01% to 98.63 ± 0.21%. The drug content in each of the film was found to be high (>90 %) suggesting a high retaining capacity of losartan potassium that will be available to permeate transbuccally. Formulation procedures involve fewer processing steps, no major drug loss was observed during the preparation of the films. All the films were found to be with uniform thickness and weight variation. The uniform thickness and weight of the films suggested the uniform distribution of drug and polymer over the surface selected for film-forming. The thicknesses of all formulated films were observed to be in the range of 0.37±0.12mm to 0.61±0.05mm and weight was found to be in the range of 19±0.61 mg to 43±0.78 mg. From this study it was observed that with increase in plasticizer concentration the thickness and weight was found to increase gradually.

Considering the fact that acidic or alkaline pH may cause irritation to the buccal mucosa and influence the degree of hydration of polymers, the surface pH of the buccal films was determined to optimize both drug permeation and mucoadhesion. The surface pH of all formulations was observed to be in the range of 6.4±0.12 to 6.9±0.27 which are within the range of salivary pH. No significant difference was found in surface pH of different films.

Swelling studies and mechanical properties of formulated losartan potassium mucoadhesive buccal films.

Hydration is required for a mucoadhesive polymer to expand and create a proper macromolecular mesh of sufficient size, and also to induce mobility in the polymer chains in order to enhance the interpenetration process between polymer and mucin. Polymer swelling permits a mechanical entanglement by exposing the bioadhesive sites for hydrogen bonding and/or electrostatic interaction between the polymer exists where optimum swelling and bioadhesion occurs. [24]

Effect of polymer concentration and composition on swelling in non-conjugated polymers

Table 4 showed the swelling index % of losartan potassium mucoadhesive films containing different bioadhesive polymers (sodium alginate, chitosan). It was found that sodium alginate films (S1 to S6) had the highest swelling index % in range of 136.24±0.32 to 153.18±0.31 after 8 h and chitosan films (C1 to C6) had the least swelling index % in range of 89.55±0.12 to 92.64±0.24 after 8 h. The low solubility of chitosan above pH 6 might have limited the swelling of films and Chitosan has a rigid structure and can allow little amount of water molecules to penetrate the matrix. The hydrophilic polymer sodium alginate increased the surface wettability and consequently water penetration within the matrix. As sodium alginate is hydrophilic polymer and has ability to absorb water so maximum swelling index % was seen with Formulations containing sodium alginate (S1 to S6) as compared with formulations containing chitosan (C1 to C6).

The rate and extent of swelling of films increased with an increasing concentration of sodium alginate. Formulation S2 containing sodium alginate 600 mg exhibited the highest swelling index 155.21±0.13 while formulation S1 having lowest concentration of sodium alginate 450 mg exhibited swelling index 136.24±0.32 after 8 h. It was found that chitosan films containing chitosan 600 mg had the swelling index 92.46±0.47 after 8 h and films containing 450 mg of chitosan had the swelling index 79.55±0.12. It was observed that the films containing more amount of polymer shows more swelling index, this would be due to more swelling of polymers and hold more amount of water in their network.

Effect of conjugation on Swelling

The effect of conjugation on the swelling behavior of various mucoadhesive polymers was observed (table 4). The conjugated polymeric films showed high swelling index in comparison to films containing non-conjugated polymers. The conjugated sodium alginate films (Cs) showed the swelling index of 298.46±0.13 after 8 h, whereas those made with pure sodium alginate (S1 to S6) showed maximum swelling index 154.46±0.12 after 8 h, respectively. The conjugated chitosan films (Cc) showed the selling index 238.43±0.21 after 8 h, whereas those made with pure chitosan (C1 to C6) showed maximum swelling index 93.68±0.16 after 8 h.

In case of conjugated sodium alginate conjugated chitosan due to presence of thiol group in place of primary amino group of chitosan the polymer chain

gets opened and forms a loose matrix which allows more number of water molecules to penetrate. Hence the swelling index of film with conjugated polymer was found to be increased compared with films contained pure polymers.[25]

Folding endurance

The folding endurance measures the breaking ability of the films during use. Sufficient folding endurance (> 300) times was found for the all formulated films of each batch, suggesting the use of the system for a period of 24 h or more without breaking or cracking (Table 4). This might be due to adequate content of glycerin which provides it with high mechanical strength and good elasticity.

Table 3: Physical characteristics of losartan potassium mucoadhesive buccal films.

Formulation	Weight (mg)	Thickness (mm)	Surface pH	Drug content (%)
S1	19±0.61	0.37±0.12	6.7±0.23	98.63±0.21
S2	22±0.48	0.41±0.24	6.6±0.25	97.34±0.07
S3	26±0.37	0.43±0.15	6.7±0.31	96.21±0.17
S4	29±0.21	0.46±0.07	6.8±0.38	98.28±0.31
S5	32±0.05	0.49±0.14	6.7±0.12	97.12±0.08
S6	36±0.14	0.51±0.08	6.6±0.35	98.29±0.47
C1	21±0.42	0.39±0.02	6.4±0.12	95.13±0.14
C2	24±0.16	0.43±0.24	6.5±0.14	94.14±0.02
C3	29±0.08	0.46±0.17	6.5±0.12	96.29±0.25
C4	33±0.67	0.48±0.21	6.4±0.26	94.03±0.13
C5	36±0.29	0.52±0.04	6.6±0.18	93.43±0.18
C6	39±0.07	0.56±0.11	6.5±0.07	95.29±0.01
Cs	41±0.54	0.59±0.24	6.9±0.27	98.21±0.24
Cc	43±0.78	0.61±0.05	6.8±0.16	97.44±0.54

Values are mean±SD, n=3

Table 4: Swelling studies and mechanical properties of losartan potassium mucoadhesive buccal films.

Formulation	Swelling Index (%) After 8 h	Bioadhesive Strength (g)	Force of Adhesion (N)	Folding Endurance	In vitro residence time (h)
S1	136.24±0.32	19.67±0.32	0.192±0.07	> 300	7.43±0.43
S2	155.21±0.13	21.83±0.43	0.214±0.01	> 300	> 8
S3	154.11±0.08	21.66±0.37	0.212±0.04	> 300	> 8
S4	154.46±0.12	22.21±0.56	0.217±0.12	> 300	> 8
S5	154.23±0.03	21.43±0.27	0.210±0.17	> 300	> 8
S6	153.18±0.31	21.33±0.16	0.209±0.08	> 300	> 8
C1	79.55±0.12	13.34±0.34	0.130±0.26	> 300	> 8
C2	92.46±0.47	14.52±0.54	0.142±0.04	> 300	6.32±0.75
C3	93.68±0.16	14.15±0.21	0.138±0.42	> 300	> 8
C4	92.91±0.02	14.42±0.19	0.141±0.18	> 300	> 8
C5	93.07±0.18	14.84±0.47	0.145±0.13	> 300	> 8
C6	92.64±0.24	14.53±0.37	0.142±0.24	> 300	> 8
Cs	298.46±0.13	32.32±0.26	0.317±0.27	> 300	> 8
Cc	238.43±0.21	28.92±0.12	0.283±0.06	> 300	> 8

Values are mean±SD, n=3

BIOADHESIVE STUDIES

Effect of polymer concentration and composition on bioadhesive strength in non-conjugated polymers

Table 4 showed the bioadhesive strength of losartan potassium mucoadhesive films containing different bioadhesive polymers (sodium alginate, chitosan). From comparision studies between these two polymers, it was found that sodium alginate films (S1 to S6) had the highest bioadhesive strength in range of 19.67±0.32 (g) to 21.33±0.16 (g) and chitosan films (C1 to C6) had the least bioadhesive strength in range of 13.34±0.34 (g) to 14.53±0.37 (g).

The polymeric nature of sodium alginate provides the polymer with unique gelling characteristics, which in turn are responsible for its adhesive properties, in addition to its rapid swelling, high mechanical strength, high water uptake, and high elasticity. Linear chains of sodium alginate exhibit strong bioadhesive behavior either because of hydrogen bonding due to hydroxyl groups or because of significant chain penetration. Chitosan is a promising bioadhesive material at neutral or slightly alkaline pH, which is found to be advantageous for adsorption on the mucosal surface. It was suggested that, at this pH, chitosan has numerous amine and hydroxyl groups as well as a number of amino groups that may increase the interaction with the negative mucin. Additionally, chitosan in general has mucoadhesive properties because of its positive charge. [26] The electrostatic attraction between the positively charged chitosan and the negatively charged mucus was demonstrated. Chitosan has less swelling and gelling properties as compared to sodium alginate this is may be the reason that the chitosan films showed less bioadhesive properties towards porcine buccal mucosa than sodium alginate films (figure 7).

Effect of conjugation on bioadhesive strength

The effect of conjugation on the bioadhesive properties of various mucoadhesive polymers was observed (table 4). The conjugated polymeric films showed high bioadhesive strength and work of adhesion in comparision to films containing non-conjugated polymers.

In Figure, the conjugated sodium alginate films (Cs) showed the bioadhesive strength (g) 32.32±0.26, whereas those made with pure sodium alginate (S1 to S6) showed maximum bioadhesive strength 22.21±0.56 (g), respectively. The conjugated chitosan films (Cc) showed the bioadhesive strength 238.43±0.21 (g), whereas those made with pure chitosan (C1 to C6) showed maximum bioadhesive strength 93.68±0.16 (g).

Bioadhesive studies carried out with alginate, chitosan and the alginate–cysteine conjugate and chitosan-thioglycolic revealed a strong influence of covalently attached cysteine and thioglycolic acid on the mucoadhesive properties of the polymer. Because of the immobilized thiol groups being capable of forming disulfide bonds with mucus glycoproteins the mucoadhesiveness of the conjugated polymer was more than that of the unmodified original polymer. The results of adhesion studies are shown in figure 7. A rapid swelling behaviour of mucoadhesive polymers favours the interdiffusion process between the polymer and the mucus layer providing stronger adhesion than it is the case for poorly swelling polymers. [27] Accordingly, the swelling behaviour has an influence on the adhesive properties of a polymer. Swelling studies were therefore carried out with the conjugated polymers and un- modified polymers. The results as shown in figure 7 demonstrate that the covalent attachment of cysteine to the polymer leads to a significant increase in the swelling behaviour of the polymer. This improved water uptake of the conjugate should contribute to the mucoadhesiveness of the thiolated polymer.

In vitro residence time

In vitro residence time data as represented in Table 4, none of the formulations were detached from the oral mucosa over the study period, which indicated that the bioadhesion values of all formulations were satisfactory to retain the film on the buccal mucosa.

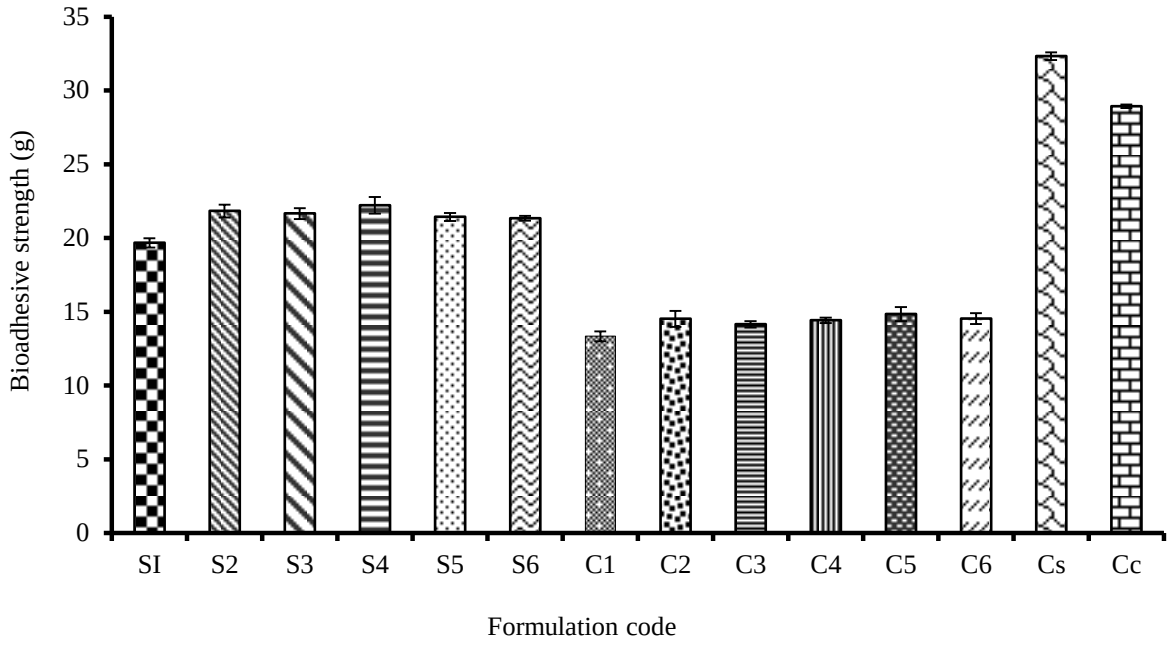

Figure 7: Bioadhesive studies of formulated losartan potassium mucoadhesive buccal films

In vitro permeation studies of formulated losartan potassium mucoadhesive buccal films

The release profile of losartan potassium films were illustrated in figures 8 and 9. The cumulative percent drug permeated from the formulations S1, S2, S3, S4, S5 and S6 was found to be 96.07±0.64%, 88.58±0.23%, 91.72±021%, 93.34±0.52%, 97.33±0.18% and 89.61±0.48%, respectively in 6 h. The cumulative percent drug permeated of films decreased with an increasing concentration of sodium alginate (figure 8). Formulation S2 containing sodium alginate 600 mg exhibited the cumulative percent drug permeation of 96.07 ±0.64 while formulation S1 having lowest concentration of sodium alginate 450 mg exhibited drug permeation of 88.58±0.23. Conversely, a higher glycerin percent increased the drug permeation, [28] particularly at a polymer concentration of 10 to 30% w/v (S2, S3, S4 and S5). A further increase of glycerin percent (40 to 50% w/v) did not improve losartan potassium permeation (S6). From drug permeation studies of the formulations S1, S2, S3, S4, S5 and S6 it was found that all the formulation showed burst drug permeation at end of 2 h followed by sustained drug permeation up to 6 h. This could be attributed to the higher rate and extent of swelling of the hydrophilic polymer, sodium alginate and also due to the hydrophilic nature of drug.

Chitosan-containing films (C1 to C6) produced sustained permeation of drug up to 8 h in all formulations (Figure 9). The cumulative percent drug permeated from the formulations C1, C2, C3, C4, C5 and C6 was found to be 57.52±0.87%, 46.49±0.64%, 48.78±0.31%, 52.34±0.47%, 50.73±0.62% and 46.98 ±0.81%, respectively in 8 h. The cumulative percent drug permeated of films decreased with an increasing concentration of chitosan (figure 9). Formulation C2 containing chitosan 600 mg exhibited the cumulative percent drug permeation of 46.49±0.64 % while formulation C1 having lowest concentration of sodium alginate 450 mg exhibited drug permeation of 57.52±0.87%. Conversely, a higher glycerin percent increased the drug permeation, particularly at a polymer concentration of 10 to 30% (w/v) (C2, C3, and C4). A further increase of glycerin percent (40 to 50% w/v) did not improve losartan potassium permeation (C5 and C6). From drug permeation studies of the formulations C1, C2, C3, C4, C5 and C6 it was found that all the formulation showed sustained drug permeation up to 8 h. This could be attributed to the low rate and extent of swelling of the water insoluble polymer, chitosan. The effect of polymer composition on drug permeation of various mucoadhesive polymers was observed (sodium alginate and chitosan). The sodium alginate films (S1 to S6) showed high drug permeation in the range of 89.61±0.48% to 97.33±0.18% with in 6 h where as

those films which containing chitosan (C1 to C6) showed sustained drug permeation in the range of 46.49±0.64% to 57.52±0.87% up to 8 h. From the release profile it is clearly evident that the drug permeation was more and quick for hydrophilic and rapid swellable polymer polymer as compare to hydrophobic and less swellable polymer chitosan (Figures 8 and 9). The effect of conjugation on the drug permeation of various mucoadhesive polymers was observed (sodium alginate and conjugated sodium alginate). The conjugated polymeric films showed sustained and slow drug release in comparisons to films containing non-conjugated polymers. In Figure 9, the conjugated sodium alginate films (Cs) showed the cumulative percent drug permeation of 82.87±0.53% after 8 h whereas those made with pure sodium alginate (S1 to S6) showed maximum cumulative percent drug permeation of 97.33±0.18% respectively, within 6 hours.(figure 12). The formation of disulfide bonds just within the thiolated polymer itself, leading also to an increase of viscosity. [29] Due to the high viscosity, high bioadhesion properties and high cohesiveness of conjugated sodium algnate (Cs) showed sustained and slow release up to 8 h. In Figure 9, the conjugated chitosan films (Cc) showed the cumulative percent drug permeation of 74.48±0.69% after 8 h whereas those made with pure chitosan (S1 to S6) showed maximum cumulative percent drug permeation of 57.52±0.43% respectively, within 8 hours. The conjugated polymeric films showed sustained and more drug release in comparision to films containing non-conjugated polymers. This is due to the property of conjugated chitosan in which the primary amino group is replaced by thiol group of thioglycolic acid, which results in opening of polymer chain and increasing swelling index, moisture content, moisture uptake, permeation coefficient and results in increased drug permeation. [30] Formulations S5, C4, Cs and Cc showed good swelling, high bioadhesive properties, a convenient residence time as well as promising drug permeation pattern. On the basis of drug permeation pattern, swelling, bioadhesion properties and residence time, S5, C4, Cs and Cc formulations were selected for *ex vivo* study.

Ex vivo permeation studies of formulated losartan potassium mucoadhesive buccal films

In *ex vivo* study, drug permeation through the goat buccal mucosa was determined for optimized formulations S5, C4, Cs and Cc. The optimized formulations S5, C4, Cs and Cc cumulative percentage drug permeation rates are 97.33±0.18%, 48.78±0.31%, 82.87±0.53% and 74.48±0.69% through the dialysis membrane and the cumulative percentage drug permeation rates through goat buccal mucosa are 92.33±0.265%, 45.78±0.52%, 77.87±0.43%, and 70.48±0.76% respectively; results of the same are shown in figures 10-13. From the results we can conclude that the permeation through the biological membrane is less than that of dialysis membrane because of the presence of epithelial membrane.

In vitro permeation studies of formulated losartan potassium mucoadhesive buccal films

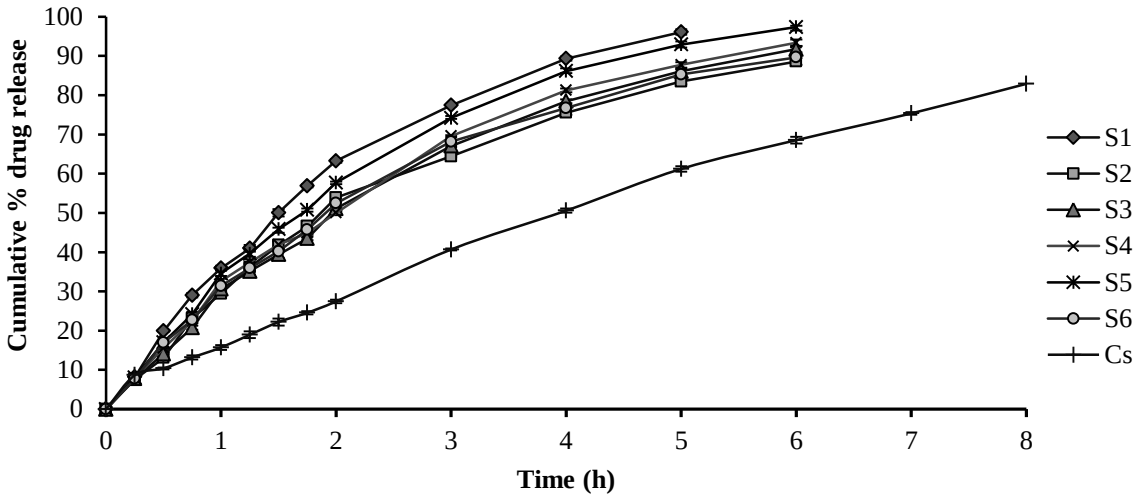

Figure 8: *In vitro* drug permeation of formulations S1 to S6 and Cs

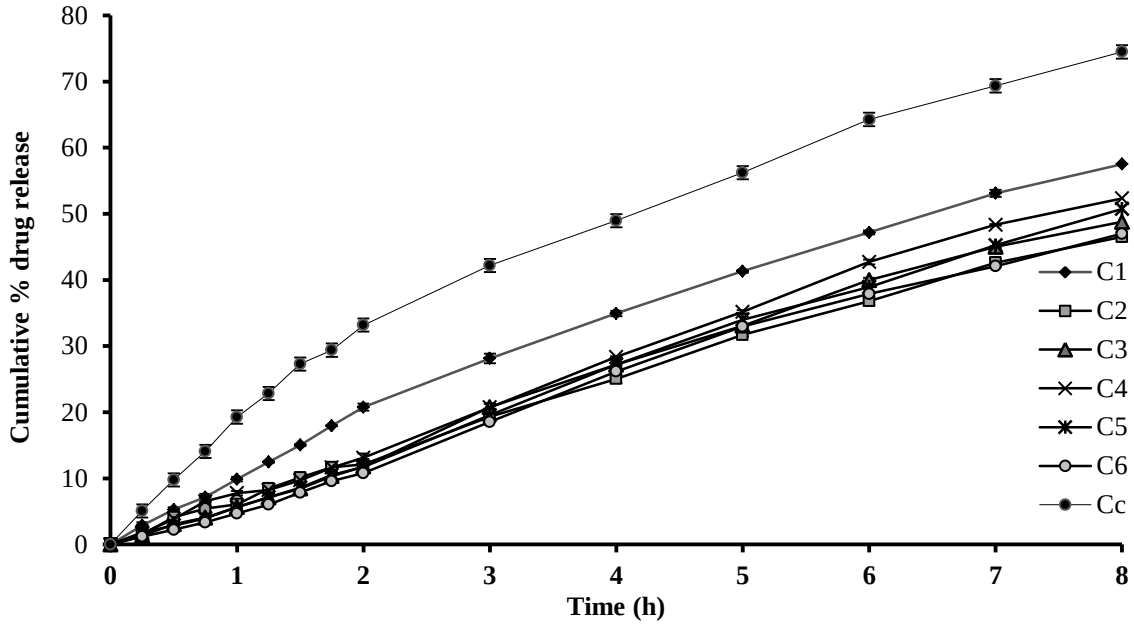

Figure 9: *In vitro* drug permeation of formulations C1 to C6 and Cs

***Ex vivo* permeation studies of formulated losartan potassium mucoadhesive buccal films**

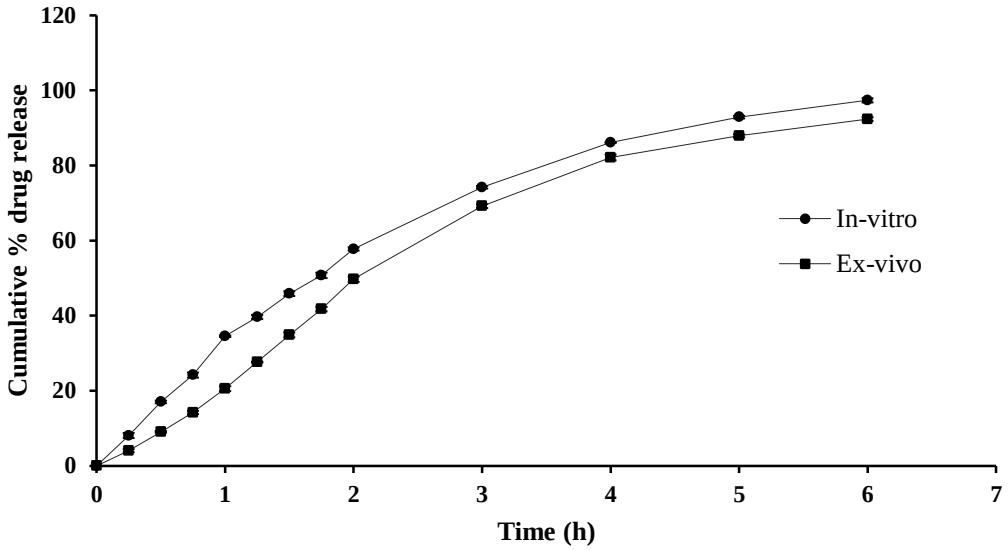

Figure 10: Comparision of *in vitro* and *ex vivo* permeation of formulation S5

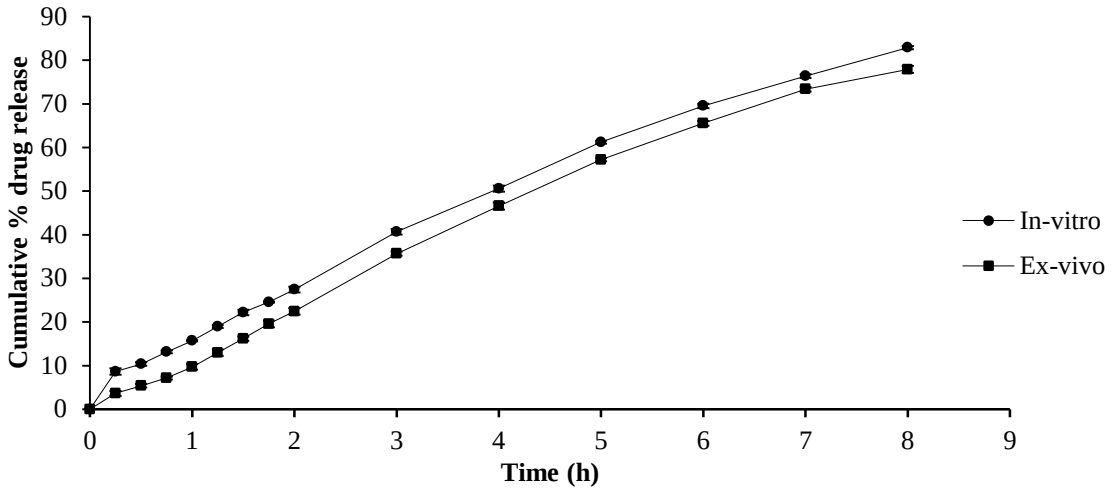

Figure 11: Comparision of *in vitro* and *ex vivo* permeation of formulation Cs

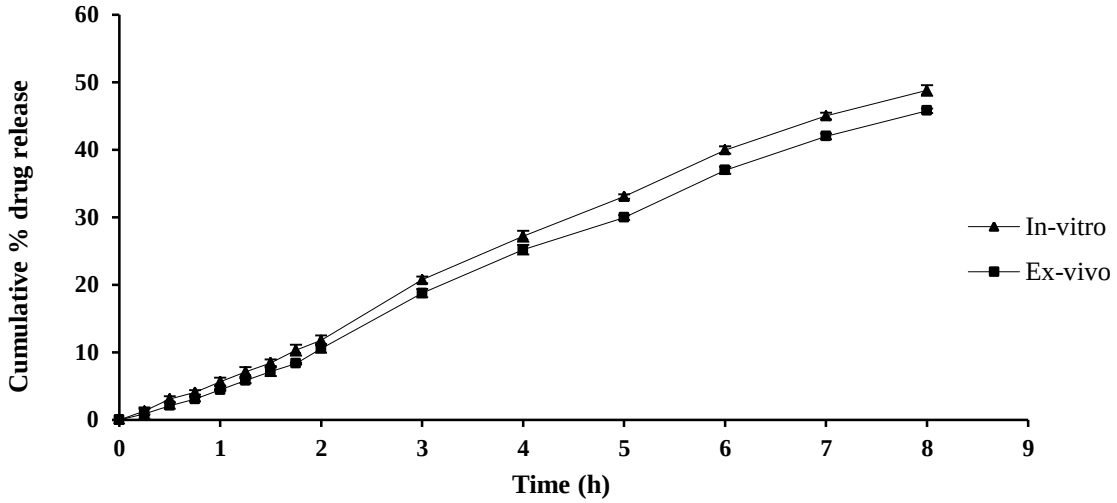

Figure 12: Comparision of *in vitro* and *ex vivo* permeation of formulation C3

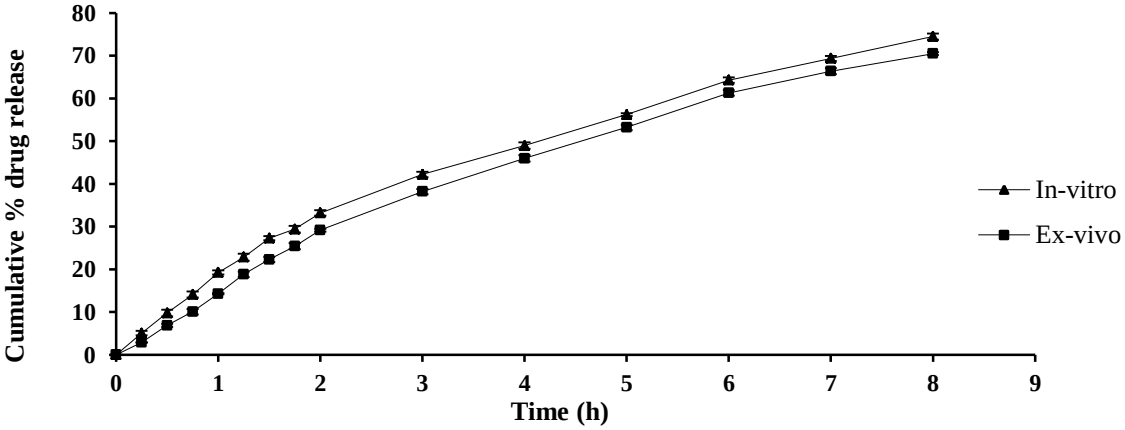

Figure 13: Comparison of *in vitro* and *ex vivo* permeation of formulation C

Kinetics of drug permeation from formulated losartan potassium mucoadhesive buccal films

Release data analysis

To analyse the mechanism of drug release from losartan potassium mucoadhesive buccal films, the drug permeation data were fitted to various kinetics models (table 5). Formulations S1 to S6 of pure sodium alginate followed first order kinetics and the formulation contains conjugated sodium alginate Cs also followed first order kinetics. Formulations C2, C3, C4 and C6 of pure chitosan followed zero order kinetics, while the formulations C1 followed first order kinetics. In case of conjugated chitosan containing formulation Cc also followed zero order kinetics.

The results of drug release mechanism are represented in Table 5. This claim was made on the basis of the n values obtained by Korsmeyer's plot, for non-Fickian release, the value of *n* falls between 0.5 and 1.0; while in the case of Fickian diffusion, *n* = 0.5; and for (super case II), *n* >1. Sodium alginate containing films (S1 to S6) showed n values in the range of 0.755 to 0.798, these values indicated the mechanism of drug permeation was found to be by non-Fickian diffusion. In case of chitosan containing films (C1 to C6) showed *n* values in the range of 1.003 to 1.050, these values indicated the mechanism of drug permeation was found to be by super case II kinetics. While in case of conjugated chitosan and conjugated sodium alginate containing films (Cs and Cc) showed *n* values 0.742 and 0.750, these values indicated the mechanism of drug permeation was found to be by non-Fickian diffusion.

Table 5: Drug release kinetics from different formulations of losartan potassium mucoadhesive buccal films.

Formulation	Zero order	First order	Higuchi	Release exponent (n)
S1	0.921	0.979	0.977	0.798
S2	0.923	0.999	0.978	0.779
S3	0.939	0.995	0.978	0.787
S4	0.934	0.993	0.979	0.773
S5	0.918	0.981	0.978	0.776
S6	0.929	0.997	0.982	0.755
C1	0.986	0.994	0.967	1.024
C2	0.997	0.996	0.934	1.003
C3	0.998	0.995	0.928	1.050
C4	0.998	0.992	0.922	1.044
C5	0.997	0.994	0.926	1.019
C6	0.994	0.995	0.926	1.046
Cs	0.988	0.987	0.965	0.742
Cc	0.957	0.996	0.988	0.750

Stability studies

Stability studies were carried out for the selected formulations (S5, C4, Cs and Cc). Results of the stability studies are shown in figures and it was found that, there was no significant difference in drug content and in vitro permeation profiles of both formulations and they were almost similar to release profiles of both formulations and they were almost similar to release profiles obtained during initial studies as shown in figures 18-21.

Table 8: Drug content of formulations S5, C4, Cs and Cc before and after stability studies

	Drug content	
Formulation	**initially**	**After stability studies**
S5	97.12±0.08	96.48±0.12
C4	94.03±0.13	92.36±0.08
Cs	98.21±0.24	97.72±0.16
Cc	97.44±0.54	96.14±0.05

CONCLUSION

The conjugation of polymers (sodium alginate-cysteine and chitosan-thioglycolic acid) were confirmed by FTIR study. Good results were obtained both *in vitro* and *ex vivo* conditions for optimized films. A higher glycerin percent in films increased the drug permeation, particularly at a polymer concentration of 10 to 30% w/v. A further increase of glycerin percent (40 to 50% w/v) did not improve losartan potassium permeation. Formulations containing conjugated sodium alginate and chitosan resulted in a significantly higher bioadhesion, swelling with better drug permeation over long period than films comprising unmodified polymers. The *ex vivo* studies has shown promising results and further *in vivo* studies need to be designed and executed to substantiate further *in vitro–in vivo* correlation.

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