Formulation and Evaluation of Cefpodoxime Proxetil Buccal Film

Ajitha K Cherian[1,*], Sr. Daisy P A[2], Noby Thomas[3], Praveen Raj[4], Liji Jacob[5], Boby Johns George[6] and Sr. Betty Carla[7]

[2]HOD, [7]Principal, [1,3,4,5,6]Department of Pharmaceutics, St. Joseph's College of Pharmacy, Cherthala, Kerala, India.

***Corresponding author:**
E-mail: ajitha.k.cherian@gmail.com

ABSTRACT

Drug delivery through the buccal mucosa offers a novel route of drug administration. Buccal films are highly flexible and readily tolerated by patients than tablets. Thus it was planned to formulate buccal films of Cefpodoxime proxetil using Chitosan, Gelatin and Pectin as main polymeric substrates. It can be used in the treatment of respiratory, urinary, skin and soft tissue infection caused by gram positive and gram negative bacteria. Various pre-formulation studies such as Infrared spectroscopy and solubility were conducted and seven formulations were prepared by solvent casting method. Physical evaluations such as physical appearance and surface texture, weight variation, mean thickness, swelling index, folding endurance, surface pH, moisture loss & moisture absorption studies and mechanical evaluations such as tensile strength, elongation at break were carried out. From the in vitro dissolution studies F6 was found to get a maximum cumulative drug release of 98.91% upto 390 minutes and selected as the optimized film containing polymers chitosan, HydroxyPropyl Methyl Cellulose, Polyvinyl Pyrrolidone and Propylene Glycol as plasticizer. Further evaluations such as anti-microbial study, stability study and release kinetics was calculated for the optimized film and is found to be within the limit.

Keywords: *Cefpodoxime proxetil, Chitosan, solvent casting method, swelling index, in vitro drug release, anti-microbial study, stability study.*

INTRODUCTION

Cefpodoxime proxetil is the orally active ester prodrug, which is absorbed and de esterified by the intestinal mucosa to release the third generation cephalosporin, Cefpodoxime. Cefpodoxime proxetil is available in oral tablet and oral suspension forms in the market. It is a molecule which has a very low solubility in water. Therefore oral bioavailability of the tablet form is only 50%. Various bio adhesive mucosal dosage forms have been developed which include adhesive tablets, gels, ointments, patches and more recently films. Buccal films are preferred over adhesive tablets in terms of flexibility and patient's comforts.

MATERIALS AND METHODS

Materials:

Cefpodoxime proxetil, as a gift sample by Sance laboratories Pvt Ltd, Kerala, Chitosan from India sea foods, Cochin, Kerala. Gelatin, Pectin, NaCMC, HPMC, PVP, PVA and PG were purchased from Chemdyes Corporation, Gujarat. Other reagents used are of analytical grade.

Methods:

Pre-Formulation Study

a) Infra- red spectroscopy[1]
b) Solubility study
c) Preparation of calibration data of Cefpodoxime proxetil using methanol[2].

Buccal films were formulated by solvent casting method, whereby the water soluble ingredients were dissolved to form a clear viscous solution and the drug (Cefpodoxime proxetil) along with other excipients dissolved in suitable solvent then both the solutions were mixed and stirred and finally casted in to the petri plate and dried. After drying the film was peeled off with a sharp blade and kept in a self-sealed cover.
Different formulations (F1 – F7) were tried using various combinations of polymers like Pectin, Gelatin, Chitosan, Sodium CMC, HPMC, Polyvinyl pyrrolidone, polyvinyl alcohol and Propylene glycol to prepare Cefpodoxime proxetil buccal film. The formulation chart is shown in Table 1.

Table 1- formulation chart

INGREDIENTS	FORMULATIONS						
	F1	F2	F3	F4	F5	F6	F7
CEFPODOXIME PROXETIL (mg)	50	50	50	50	50	50	50
PECTIN (% w/v)	4	4	-	-	-	-	-
GELATIN (% w/v)	-	-	3	3	-	-	-
CHITOSAN (% w/v)	-	-	-	-	2	2	2
SODIUM CMC (% w/v)	1.5	-	1.5	-	-	-	-
HPMC (% w/v)	-	1.5	-	1.5	-	1.5	1.5
POLYVINYL PYRROLIDONE (% w/v)	-	-	-	-	1	1	-
POLYVINYL ALCOHOL (% w/v)	-	-	-	-	1	-	1
PROPYLENE GLYCOL (% v/v)	5	5	5	5	5	5	5
PEPPERMINT FLAVOR (% v/v)	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Pectin films (F1-F2)[3]: NaCMC (1.5 % w/v) were dispersed in 3/4 the volume of distilled water at 25 °C. Then, the rest1/4 of volume distilled water was added. For F2, HPMC was dispersed in 1/3 the volume of the distilled water at 90 °C. Then, the 2/3 volume of the distilled water at 5°C was added. Pectin (4% w/v) was dispersed in dilute solution of 0.1N HCl at pH 3. Then, calcium chloride (0.1%w/v) was added and the solution was heated at 50°C. Then, plasticizer (Propylene Glycol, 5% w/v) and drug, Cefpodoxime proxetil (50mg) dissolved in methanol were blended to the polymeric solution. The medicated gel was kept overnight at room temperature to obtain clear and bubble free gel. After that, this gel will be poured to the glass petri dishes to be dried in oven at 60-70°C.

Gelatin films (F3-F4)[4]: All ingredients were accurately weighed and mixed by trituration in glass pestle and mortar. The mixture was then added gradually to magnetically stirred solvent system (distilled water) containing the plasticizer. Stirring was continued until a clear solution was obtained. Films containing Cefpodoxime proxetil were prepared by dissolving thecalculated amount of drug (50mg) in 5ml methanol. The drug solution was added to the polymer solution under stirring. The solution was then transferred quantitatively to petri-dish.The petri-dishes were covered with inverted funnels to allow controlled evaporation of the solvents. These were left undisturbed upon temperature (20-25^{0}C) for one to two days depending upon the solvent system used.

Chitosan films (F5-F7)[5,6]: 2%w/v of chitosan solution was prepared by dissolving chitosan in 1.5 % acetic acid, stirred for 2 hours. This solution was filtered through a muslin cloth to remove debris. To this polymeric solution, rest of the ingredients were added and stirred for 2 hours. This polymeric solution was kept overnight to remove air bubbles, and then it was added uniformly to a petri plate. The plate was then kept in an oven at 45^{0}C for 4 hours.
After drying, the film was peeled off with a sharp blade and kept in a self-sealed cover. Peppermint flavour (1.5 %v/v) is also added to all the above formulations to improve the taste of formulation.

Evaluation of Physical and Mechanical Characteristics of the Prepared Films

a) Physical Evaluation:
   I) Physical appearance and surface texture
   II) Weight variation
   III) Mean thickness
   IV) Swelling index
   V) Folding endurance
   VI) Surface pH
   VII) Moisture loss& moisture absorption studies
b) Mechanical Evaluation:
   I) Tensile strength
   II) Elongation at break

a) Physical Evaluation

The buccal films were evaluated for the following properties:

I) Physical Appearance and Surface Texture

Physical appearance and surface texture evaluation includes visual inspection and evaluation of texture by feel or touch.[7]

II) Weight variation

Ten films of 1cm^2 were weighed individually and average of those films measured.[8]

III) Thickness[1]

The thickness of the film was measured using screw gauge with a least count of 0.01 mm at different spots of the films. The thickness was measured at five different spots of the film and average was taken.

IV) Percent Swelling Index (Diameter method)[9]

The polymeric filmswere cut in to small film of 1.5 cm diameter. This film was placed on

the surface of the agar plate and the diameter at different time intervals were taken up to 5 hrs and the percentage swelling index was calculated using the formula,

$$SD\% = \frac{Dt - Do \times 100}{Do}$$

SD% = % swelling by diameter method
Dt = diameter of swollen film after time, t
Do = original film diameter.

V) Folding Endurance [9,7]

Folding endurance of the film was determined by repeatedly folding a small strip of the film (approximately 2x2 cm) at the same place till it broke. The number of times film could be folded at the same place, without breaking gives the value of folding endurance.

VI) Surface pH [1]

Buccal films were left to swell for 1 hour on the surface of the agar plate, the agar plate prepared by dissolving 2% (w/v) agar in warmed isotonic phosphate buffer of pH 6.6 under stirring and the solution was poured into a petri dish and was allowed to stand until solidified to form a gel at room temperature. The surface pH was measured by means of pH paper placed on the surface of the swollen patch.

VII) Moisture loss& moisture absorption studies[10]

a. Moisture loss

The buccal films were weighed accurately and kept in desiccators containing anhydrous calcium chloride. After three days, the films were taken out and weighed. The moisture loss (%) was determined by calculating moisture loss using the formula:

$$\text{Moisture loss (\%)} = \frac{\text{Initial weight} - \text{Final weight} \times 100}{\text{Initial weight}}$$

b. Moisture absorption

The buccal films were weighed accurately and placed in the desiccators containing 100ml of saturated solution of aluminium chloride, which maintains 76% relative humidity (RH). After three days, the films were taken out and weighed. The percentage moisture absorption was calculated using the formula:

$$\text{Moisture absorption (\%)} = \frac{\text{Final weight} - \text{Initial weight} \times 100}{\text{Initial weight}}$$

I) Mechanical Evaluation

Tensile Strength[11]

The tensile strength of buccal films refers to tension or force required to tear of the patch apart into two pieces. Tensile strength was determined using an instrument assembled in the laboratory.

Instrument

The instrument used to measure the tensile strength designed in our laboratory especially for this project work. The instrument was designed in such a way that the film can be fixed up between two hooks horizontally to hold the test film. A film of 2.5cm length was attached to one side hook of the balance and the other side hook was attached to plate fixed up to the pan. The arrangement is shown in Figure 1.

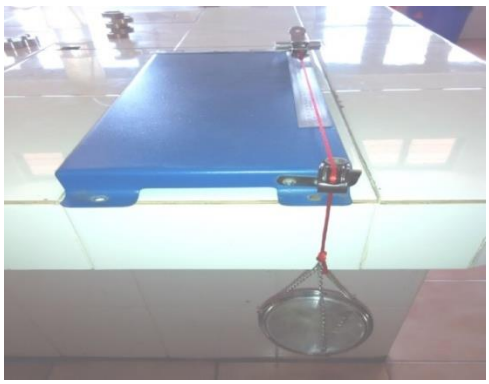

Figure.1: Modified instrument used for the measurement of tensile strength.

Method of Calculation

The definition of tensile strength as per American Standard for Testing Material (ASTM) standard tests principles is, "the maximum load during the tensile strength test divided by the original minimum cross-sectional area of the specimen". Thus, tensile strength,

$$T = \frac{m \times g\ N/m^2}{b \times t}$$

Where,
m = mass in Kg
g = acceleration due to gravity 9.8m/sec²
b = breadth of the specimen in m
t = thickness of sample in m.

T= force at break/ initial cross-sectional area of sample.

ii) Percent Elongation at Break[12]

The percent elongation at break is defined as the elongation at the momentof rupture of the specimen divided by the initial gauge length of the specimen and multiplying by 100.

$$\text{Percent elongation at break} = \frac{LB - L0 \times 100}{L0}$$

LB = length of the specimen in cm when it breaks.
L0 = original length of the specimen in cm.

Evaluation of Cefpodoxime Films.

i) Drug Content Determination[13]

The weight of whole film was determined. For determining the drug content, a single piece of film was taken and crushed in a mortar using pestle. Methanol was added and triturated to completely dissolve the drug; it was then diluted to 100ml. The solution was filtered. The absorbance of the solution was measured using UV spectrophotometer at 235 nm and the drug loading was calculated.The polymer solution without drug serves as a blank. Percentage drug loading was calculated using formula.

$$\% \text{ drug loading} = \frac{\text{Practical loading} \times 100}{\text{Theoretical drug loading}}$$

ii) *In vitro* release study[5]

The *in vitro* release study was carried out using USP dissolution apparatus type 2 in 400ml phosphate buffer pH 6.8 at 50 rpm and a temperature of 37 ± 0.5°C. A film was taken and attached to a glass slide in order to prevent floating of film over the dissolution media. The *in vitro* release study was carried out for 7 hours. 5ml of samples were withdrawn at various times interval, replacing with fresh medium each interval, absorbance of the samples were measured at 235 nm, and the cumulative percentage release was calculated.

Further evaluation of the optimized film is done in the following headings:

1. Release Kinetics[14]

i) Determination of order of release of drug from buccal film by Graphical method

To determine the order of release of drug from buccal film by graphical method using the dissolution data, a graph was plotted with cumulative % drug release vs time. Zero order release can be confirmed if a straight line or linearity is obtained. Regression coefficient of the curve was determined to confirm the correlation between X and Y.

ii) Mechanism of drug release study

In order to predict and correlate the release behaviour of drug from the buccal film, it is necessary to fit the *in vitro* release data into a suitable model. A graph was plotted with cumulative % drug release vs. √time. If linearity is observed, the release mechanism is by Higuchi's diffusion.

2. Determination of Bioadhesive Strength of Optimized Films

Measurement of Bioadhesive Strength[15]

The tensile strength required to detach the polymeric film from the mucosal surface was applied as a measure of the bioadhesive performance.

Instrument

The apparatus was locally assembled and was a modification of the physical balance. The device was mainly composed of a two-arm balance. The left arm of the balance was replaced by small stainless steel lamina vertically suspended. At the same side, a platform was maintained in the bottom in order to fix the model mucosal membrane.

Method

The device was mainly composed of a two-arm balance. The left arm of the balance was replaced by small stainless steel lamina vertically suspended. At the same side, a platform was maintained in the bottom in order to fix the model mucosal membrane. The bovine cheek pouch excised and washed was fixed to the platform. The mucoadhesive patch was fixed of 3cm², was fixed to the stainless steel lamina using an adhesive. The exposed patch surface was moistened with 1ml of isotonic phosphate buffer for 30 seconds for initial hydration and swelling. The platform was then raised upward until the hydrated patch was brought into the contact with the mucosal surface.

A preload of 20gms was placed over the stainless steel lamina for 3 minutes as initial pressure. And then weights were slowly increased on the right pan, till the patch

detaches from the mucosal membrane. Force required detaching the patch from the mucosa give the bioadhesive strength of the mucoadhesive patch. The procedure is repeated for 3 times for each patch and mean value of the 3-trials was taken for each set of formulation. After each measurement the tissue was gently and thoroughly washed with isotonic phosphate buffer and left for 5 minutes before taking reading.

$$\text{Bioadhesive force, } F = \frac{(W_w \times g)}{A}$$

Where,
W_w –Weight applied (g)
g- Acceleration due to gravity (cm/ s^2)
A - Surface area of the patch (cm^2)

3. Antibacterial Study of Optimized Film[16]

The agar plates used in this study were prepared by dissolving nutrient agar 28 g in 1 L of distilled water and sterilized by autoclaving (at 15 lb pressure and 121 °C) for 15 min. The agar solution was poured into sterile Petri dishes. The agar plates were then allowed to cool and solidify at room temperature; then they were inoculated (cultured) with *S. aureus* and *E.coli*.

Agar Diffusion Assay

Antibacterial efficacy of the optimized buccal film of Cefpodoxime (F6) was determined by subjecting the aliquot of *in-vitro* drug release studies to agar diffusion assay. Aliquot of *in-vitro*drug release sample was collected after 6.5 h. A 0.1 mL sample was carefully pipetted into uniformly spaced wells of the agar plates. Reference standard also prepared using pure Cefpodoxime proxetil and inoculated in an identical condition. These plates were allowed to prediffuse for 2 hat cold temperature and then incubated for 24 h. The diameter (millimeter) of the growth inhibition zone surrounding each agar well inoculated with *S.aureus* and *E.coli* were measured.

4. Stability Study of the Optimized Film[1]

Optimized medicated films were subjected to stability testing. Films were placed in a beaker lined with aluminium foil and kept in a humidity chamber maintained at 40±2^{0}C and 75±5% relative humidity for 1 month. Changes in the appearance, drug content and *In-vitro* release of the stored films were investigated at the end of 15 days and 30 days.

RESULTS AND DISCUSSION

Pre formulation studies:

a) IR spectroscopic studies

IR spectrum for pure drug and physical mixture of drug-polymers were obtained and analyzed for principle peaks. These peaks can be considered as characteristic peaks of Cefpodoxime proxetil and were not affected and prominently observed in IR spectra of drug along with polymers such as pectin, chitosan, gelatin, sodium CMC, HPMC, PVP and PVA. The spectra indicated no interaction between Cefpodoxime proxetil and the selected polymers. So the drug and polymers are compatible with each other.

b) Solubility studies

Cefpodoxime Proxetil was found to be insoluble in water, slightly soluble in ether, freely soluble in acetonitrile, isopropyl alcohol, methanol and DMSO. Solubility study has been performed for Cefpodoxime proxetil and the results were within the pharmacopoeial specifications.

c) Preparation of calibration data of cefpodoxime proxetil Using methanol

The absorption readings of Cefpodoxime proxetil solution (25-150µg/ml) in methanol at the maximum wavelength of 235 nm were tabulated in table 7.2 and figure 7.2 shows a calibration curve for the readings. The curve was found to be linear with in the concentration range of 25-150 µg/ml. the calibration data is given in Table 2 and the curve shown in Figure 2.

Table: 2 - Calibration data of Cefpodoxime proxetil using methanol

Concentration (µg/ml)	Absorbanceat 235nm
0	0
25	0.2174
50	0.4231
75	0.6433
100	0.8639
125	1.0580
150	1.2860

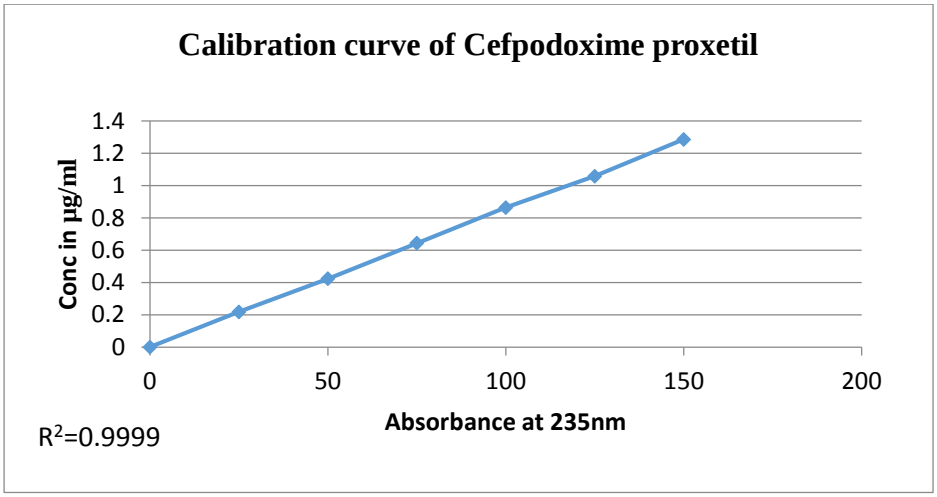

Figure 2- Calibration curve of Cefpodoxime using methanol.

Evaluation of Physical and Mechanical Characteristics of the Prepared Films

Physical evaluation

The seven prepared buccal films were evaluated for different physical parameters and the results were recorded in table 3.

Table 3 -Physical evaluation data of formulations

Physical characteristics	Formulations						
	F1	F2	F3	F4	F5	F6	F7
Appearance	Less smooth	smooth	Less smooth	smooth	smooth	smooth	Smooth
Surface texture	Less flexible	Flexible	Less flexible	flexible	Flexible	Flexible	Flexible
Thickness (mm)	0.403 ±0.14	0.402 ±0.08	0.402 ±0.09	0.402 ±0.09	0.401 ±0.11	0.402 ±0.07	0.402 ±0.09
Average Weight (mg)	55 ±0.95	56 ±1.07	58 ±1.24	57 ±1.73	58 ±1.44	58 ±0.95	56 ±1.05
Swelling index after 6hr (%)	27.05 ±0.12	28.06 ±0.22	27.13 ±0.24	28.11 ±0.31	26.51 ±0.26	28.14 ±0.31	28.01 ±0.18
Folding endurance	291 ±5	>300	>300	>300	>300	>300	>300
Surface pH	7	7	7	7	7	7	7
Moisture absorption (%)	4.35 ±0.01	4.15 ±0.03	5.92 ±0.02	5.64 ±0.03	5.35 ±0.01	5.16 ±0.02	5.94 ±0.03
Moisture loss (%)	1.85 ±0.08	1.88 ±0.06	1.91 ±0.03	1.94 ±0.04	1.48 ±0.05	2.02 ±0.04	2.09 ±0.06

By the physical evaluations better appearance and texture was showed by all the seven formulations. Formulations F1 & F3 showed comparatively less smooth surface and flexibility. Physical appearance

helps in improving the patient compliance to some extent.

The average weight and thickness of all the films were taken and results given on the table. The average weight of the formulations were from 55±0.95 to 58±1.44 mg and the thickness are in the range of 0.401±0.11 to 0.403±0.14.Results showed that thickness and weight of all the formulations are in the acceptable limit. This ensures a uniform release of drug during release studies.

Swelling studies of different films showed that all the formulated films are in the standard limits. Formulations F2, F4, F6 & F7 showed comparatively similar values of swelling index i.e. around 28 %.F5 having least swelling index amongst all other films with around 26.5 %. Increased swelling index may be due to the presence of swellable polymer like HPMC.

The optimum folding endurance for buccal film is above 300. Folding endurance of seven different buccal films showed that formulationF1 having least folding endurance with 291 times. Remaining 6 formulations with a folding endurance value greater than 300 which is ideal for buccal films.

The surface pH of all the formulations was 7 i.e. neutral pH and hence no irritation was expected during buccal administration.

The percentage moisture absorption were found out and tabulated. This varies from 4.15±0.03 to 5.94±0.03 %. The percentage moisture loss of all the 7 formulations were calculated and found to be in the range of 1.48±0.05 to 2.09±0.06. All the tested formulations from F1 to F7 showed minimum moisture loss which is helpful for maintaining the flexibility and other physical characters of the film during storage.

Physico-chemical parameters of all antibiotic buccal film formulations were found to be within acceptable limit.

Mechanical evaluation

For mechanical evaluation tensile strength and percentage elongation at break were studied and the results were tabulated in table 4

Table 4 – Mechanical evaluation data of formulations

Formulations	Tensile strength (N/m^2)	% Elongation at break
F1	$4.6 \times 10^3 \pm 0.14 \times 10^3$	44.1 ± 1.9
F2	$4.7 \times 10^3 \pm 0.12 \times 10^3$	46.3 ± 1.5
F3	$4.6 \times 10^3 \pm 0.16 \times 10^3$	48.1 ± 1.9
F4	$4.9 \times 10^3 \pm 0.14 \times 10^3$	47.8 ± 1.8
F5	$5.4 \times 10^3 \pm 0.13 \times 10^3$	50.4 ± 1.1
F6	$5.6 \times 10^3 \pm 0.15 \times 10^3$	49.3 ± 1.8
F7	$5.1 \times 10^3 \pm 0.11 \times 10^3$	48.6 ± 1.6

Film F7 showed highest tensile strength of $5.6 \times 10^3 \pm 0.15 \times 10^3 N/m^2$, this indicates the combination produce effective cross-linking. The values of other formulations were also found to be in the range of buccal films.

The elongation at break test provides an indication of the strength and elasticity of the film which is reflected by the elongation of the break. Films suitable for buccal application should preferably be strong but flexible. The evaluation of different formulations showed highest elongation is observed in formulation F5, 50.4 ± 1.1%.The values of other formulations are near to the range and satisfactory.

Drug Content Determination

The drug loading efficiency of all formulations was studied and result showed that the drug content of all formulations were uniform which ranges between 98.16 and 98.88 %.Highest drug loading was found to be produced by formulationF4 and F6. Lowest drug loading was produced by formulation F1. The combination of Chitosan and HPMC is giving a promising drug content in its formulations. The chart is shown in Table 5.

Table 5 – Percent drug content of formulations

FORMULATIONS	DRUG CONTENT (%)
F1	98.16 ± 0.14
F2	98.39 ± 0.21
F3	98.27 ± 0.19
F4	98.88 ± 0.01
F5	98.16 ± 0.24
F6	98.88 ± 0.03
F7	98.51 ± 0.16

In Vitro Drug Release

The release of drug from the dosage form plays important role in buccal drug delivery and in determining the therapeutic effect of the medication. The *in vitro* release study of all formulations of buccal film was carried out using USP dissolution apparatus type 2 in 400 ml phosphate buffer pH 6.8 at 50 rpm. A comparison of the 7 *in vitro* drug release is shown in table 6 and figure 3.

Table 6 - Comparison of *In vitro* drug release of formulations F1-F7

Time in minutes	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
30	33.6	15.5	24	10.5	10.75	9	9.5
60	69.49	32.98	43.56	21.99	22.00	16.08	16.11
90	96.26	49.17	68.14	32.18	35.12	24.65	26.11
120	96.54	69.70	85.79	45.70	45.77	29.83	35.24
150		83.83	98.87	52.14	52.37	40.73	43.80
180		96.86		62.83	68.26	45.89	49.39
210		98.25		76.32	75.90	52.37	60.32
240				88.36	90.34	63.07	68.38
270				97.41	97.43	68.42	85.30
300						75.90	91.93
330						83.90	97.44
360						90.43	98.98
390						98.91	

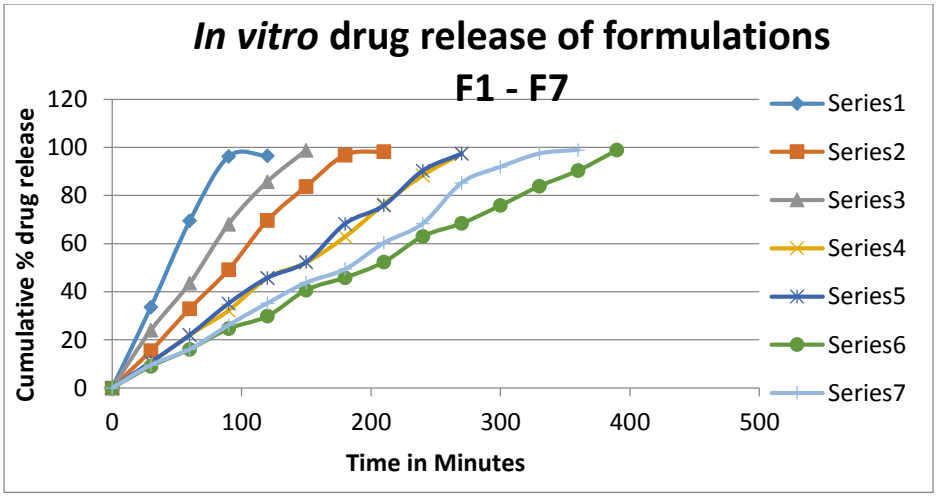

Figure 3 - Comparison of *In vitro* drug release graph of formulations F1-F7

From the above *in vitro* release studies the formulation F6 with Chitosan, HPMC and PVP can be considered as the optimized formulation due to maximum release for an extended period of 390 minutes. The graph plotted with Cumulative % drug release vs. time shows linearity in release. So this combination was found to be ideal for buccal release. The optimized formulation F6 was then considered for release kinetics and future studies.

Release Kinetics

Release kinetic study of optimized film (F6)

To determine the order of release of drug from buccal film by graphical method from the dissolution data, a graph was plotted with cumulative % drug released vs. time. Zero order release was confirmed if a straight line or linearity is obtained. Regression coefficient of the curve was determined to confirm the correlation between X and Y. The zero order release was confirmed for formulation F6 as it gave a straight line with r^2 value of 0.9985 when plotted with cumulative % drug released vs. time. The graph and linearity is shown in figure 4.

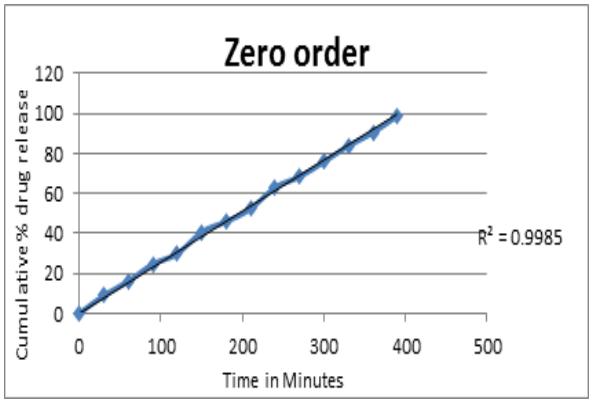

Figure 4: Release kinetics of formulation F6

Mechanism of Drug release of optimized film (F6)

In order to predict and correlate the release behavior of drug from the buccal film, a graph was plotted with cumulative % drug release vs. √time, figure 5.

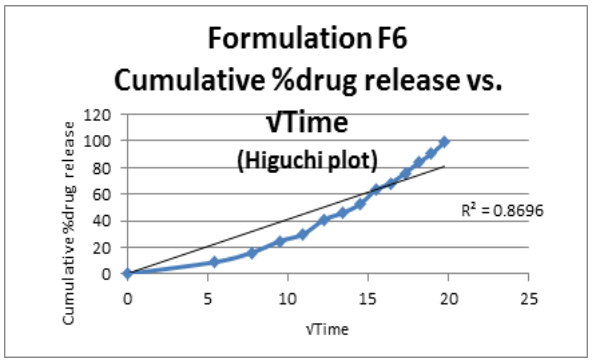

Figure 5 -Mechanism of Drug release of formulation F6

From the data shown in Table 7, the *in vitro* dissolution data were fit in to Higuchi's diffusion plot with r^2 value 0.8696.from the result it is confirm that the prepared buccal film model for antibiotic Cefpodoxime proxetil follows zero order diffusion kinetics.

Table 7 – Higuchi plot Formulation F6

√Time	Cumulative %drug release
0	0
5.47	9
7.75	16.08
9.49	24.65
10.95	29.83
12.25	40.73
13.42	45.89
14.49	52.37
15.49	63.07
16.43	68.42
17.32	75.90
18.17	83.90
18.97	90.43
19.75	98.91

Determination of Bioadhesive Strength of Optimized Films

In general, the strength of mucoadhesion is affected by various factors such as contact time with mucus, swelling rate of the polymer and the biological membrane used in the study. An acceptable bio adhesive strength of 6.1±0.08 N was obtained for the optimized formulation of buccal film as shown in table 8.

Table: 8 – Bioadhesive strength of optimized film

Formulation	Bioadhesive strength(N)
F6	6.1±0.08

Antibacterial Study of Optimized Films

After 24 hrs of incubation the diameter of zone of inhibition was measured and the values were shown in the table 9.

Table 9 – Antibacterial study of formulation F6

Organism used	Zone of inhibition (mm)	
	Formulation - F6	Standard Cefpodoxime
E.coli	23 ± 0.08	24 ± 0.02
S.aureus	26 ± 0.06	28 ± 0.04

The drug extracted from buccal film showed better antimicrobial activity against the tested microorganisms. The zone diameter

for formulation F6 against *E.coli* was 23 ± 0.08 mm (figure 6) and against *S.aureus* was 26 ± 0.06 mm (figure 7), which is more or less equal to the zone diameter produced by pure cefpodoxime drug against the microorganisms. This clearly indicated that the entrapment of Cefpodoxime proxetil in to the buccal film makes no change in its potency and activity.

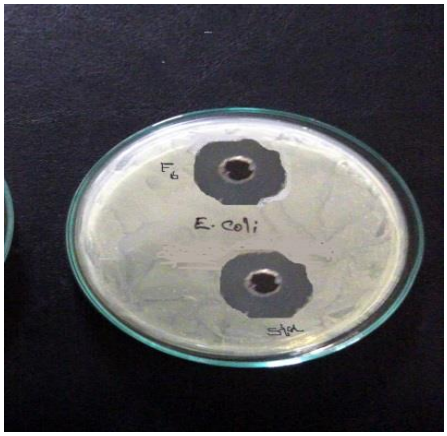

Figure 6 – Zone of inhibition against *E.coli*

Figure 7 – Zone of inhibition against *S.aureus*

Stability Study of the Optimized Film

Films of formulation F6 were kept in a humidity chamber maintained at 40±2^{0}C and 75±5% relative humidity for 1 month. Changes in the appearance and drug content of the stored films were investigated at the end of 15 days and 30 days. The readings were tabulated in table 10. From the data analyzed it was clear that the physical and mechanical evaluation doesn't show significant changes in the values. The values considered were appearance, surface texture, thickness, average weight, swelling index, folding endurance, surface pH, moisture absorption, tensile strength and % elongation at break. The values were seems to be normal even after 30 days of study. The results of *invitro* drug release study and the graphs were prepared for optimized film after 15 days and 30 days of interval and shown in (table 11 & figure 8) & (table 12 & figure 9) respectively.

Table 10 – Stability study evaluation of formulation F6

Evaluation Parameters		**After 15 days Formulation F6**	**After 30 days Formulation F6**
Physical characteristics	Appearance	Clear, smooth	Clear, smooth
	Surface texture	Flexible	Flexible
	Thickness (mm)	0.402 ± 0.14	0.402 ± 0.11
	Average Weight (mg)	58 ± 0.74	58 ± 0.61
	Swelling index after 6hr (%)	26.15 ± 0.12	26.11 ± 0.22
	Folding endurance	>300	>300
	Surface pH	7	7
	Moisture absorption (%)	5.15 ± 0.05	5.13 ± 0.02
	Moisture loss (%)	2.02 ±0.02	2.02 ±0.06
Mechanical evaluation	Tensile strength (N/m^2)	$5.6 \times 10^3 \pm 0.02 \times 10^3$	$5.5 \times 10^3 \pm 0.10 \times 10^3$
	% Elongation at break	49.2 ± 0.29	49.2 ± 0.91
Drug content determination (%)		98.46 ± 0.03	98.01 ± 0.12
***In vitro* drug release**	% Cumulative drug release	98.90	97.44
	Time (min)	390	390

Table 11 - *In vitro* drug release of formulation F6 after 15 days of stability study

Time (minutes)	**Absorbance 235nm**	**Amount of drug release (mg)**	**% drug release**	**Cumulative % drug release**
0	0	0	0	0
60	0.171	8	16	16
120	0.350	15.75	31.5	32.01
180	0.502	23.5	47	47.67
240	0.669	31.12	62.24	63.00
300	0.809	37.75	75.5	76.32
360	0.960	44.25	88.5	89.35
390	1.054	49	98	98.90

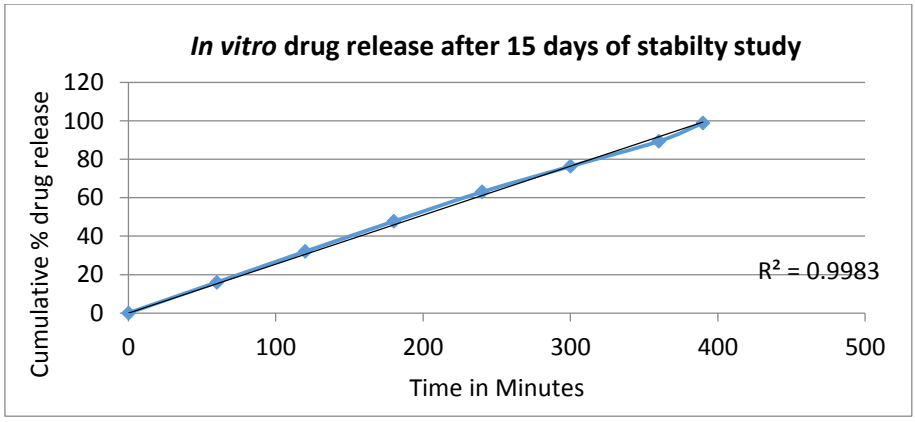

Figure 8 - *In vitro* drug release of formulation F6 after 15 days of stability study

Table 12 - *In vitro* drug release of formulation F6 after 30 days of stability study

Time (minutes)	**Absorbance 235nm**	**Amount of drug release (mg)**	**% drug release**	**Cumulative % drug release**
0	0	0	0	0
60	0.173	8	16	16
120	0.355	16.6	33.2	33.68
180	0.499	23.5	47	47.71
240	0.672	31.12	62.24	63.00
300	0.819	38.25	76.5	77.31
360	0.977	45.5	91	91.84
390	1.036	48.25	96.5	97.44

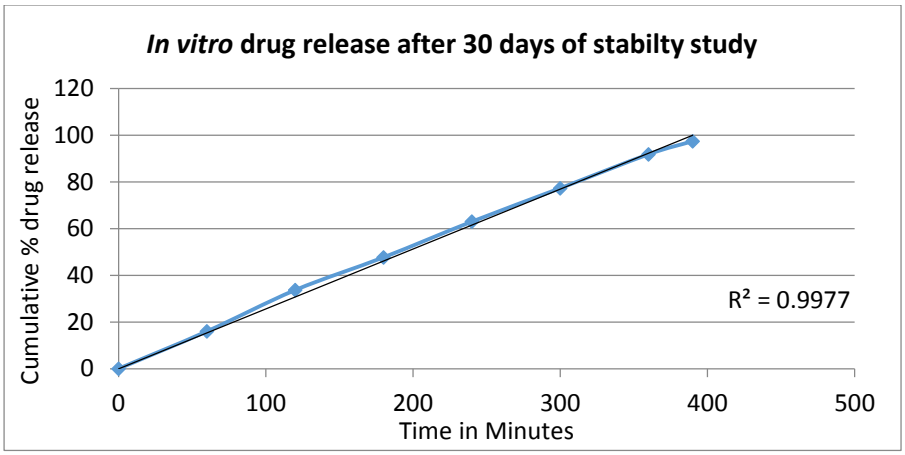

Figure 9- *In vitro* drug release of formulation F6 after 30 days of stability study

The cumulative percentage release showed after 15 days was 98.90 and after 30 days was 97.44 at 390 minute. The % release data such as time of release and Cumulative percentage release shows no significant variation from the previous values.

CONCLUSION

From the pre formulation studies, physico chemical, mechanical evaluations, *in vitro* release and stability studies conducted, formulation F6 with Chitosan, HPMC and PVP was found to be an optimized combination for antibiotic buccal film. The anti-bacterial study also reveals that this particular combination of formulation makes no problem to carry an antibiotic in to the buccal film. So the aim of the present study to formulate an antibiotic buccal film achieved. In future this system can be further developed with varying percentages of polymers for a better controlled release and bio availability. An *in vivo* study can also be conducted with suitable animal model.

ACKNOWLEDGEMENTS

Thanks to department of pharmaceutics, St Joseph's College of Pharmacy, who provided us with valuable information during the experiment. My acknowledgements to all people who helped us to carry out and finish this work.

REFERENCES:

1. J. Thimmasetty, G. S. Pandey and P. R. SatheshBabu, Design and Evaluation of Carvidilol Buccal Mucoadhesive Patches, Pakistan Journal of pharmaceutical Science. 21(3) (2008) 241-248.
2. SiddalingaSwamy M.S., Dr. A Sathish Kumar Shetty, Anil kumar S.M., UV-Visible Spectrophotometric Methods For The Estimation Of CefpodoximeProxetil In Bulk Drug And Pharmaceutical Dosage Form, International Journal of PharmTech Research, 4 (2) (2012) 750-756.
3. R. Gardouh1, M. M. Ghorab1, S. S. Badawy2 and R. B. Gales2, Preparation and Characterization of Mucoadhesive Buccal Film for Delivery of Meloxicam, British Journal of Pharmaceutical Research, 3(4)(2013) 743-766.
4. AmitKhairnar, Parridhi Jain1, DheerajBaviskar, Dinesh Jain, Developmement of mucoadhesive buccal patch containing aceclofenac: in vitro evaluations, International Journal of PharmTech Research 1(4) (2009) 978-981.
5. Subhash V. Deshmane, Madhuri A. Channawar, Anil V. Chandewar1, UnmeshM.Joshi, Kailash R. Biyani, Chitosan based sustained release mucoadhesive buccal patchescontaining verapamil HCl, International Journal of pharmacy and Pharmaceutical Sciences. 1(1) (2009) 216-227.
6. Vishnu M. Patel, Bhupendra G. Prajapati, Madhabhai M. Patel, Design and in vitro characterization of eudragit containing mucoadhesive buccal patches, International Journal of PharmTech Research. 1(3) (2009) 783-789.
7. Parikh BhavikAnjankumar, Design and evaluation of buccal patches of valsartan, IJPI's Journal of Pharmaceutics and Cosmetology, 1(2) (2011) 51-54.
8. Supriya S. Shidhaye, Nilesh S. Saindane, SagarSutar, and Vilasrao Kadam1, MucoadhesiveBilayered Patches for Administration of Sumatriptan Succinate, American Association of Pharmaceutical Scientists. 9(3) (2008) 909-916.
9. Umesh. D. Shivhare, Parag. D. Bodkhe, KishorP.Bhusari, Vijay B. Mathur, Formulation and Evaluation of Buccoadhesive Films of Losartan Potassium, Der Pharmacia Lettre.2(5) (2010) 251-260.

10. M Nappinnai, R chandanbala, R Balajirajan, Formulation and evaluation of nitrendipine buccal films, Indian Journal of Pharmaceutical Sciences. 70(5) (2008) 631-635.
11. J. Varshosaz, Z. Dehghan, Development and characterization of buccoadhesivenifedipine tablets, European Journal of Pharmaceutics and Biopharmaceutics. 54 (2002) 135–141.
12. Chinna Reddy Palem, Ramesh Gannu, Vamshi Vishnu Yamsani, Shravan Kumar Yamsani and MadhusudanRaoYamsani, Development of bilayeredmucoadhesive patches for buccal delivery of felodipine: in vitro and ex vivo characterization, Current trends in Biotechnology and Pharmacy. 4(2) (2010) 7-17.
13. Mohammed Gulzar Ahmed, G.B Kiran Kumar, and B.P. Satish Kumar, Formulation and Evaluation of Nifedipine Transdermal Patches, Journal of Pharmacy Research. 3(8) (2010) 1785-1787.
14. Umadevi S., BeethaRohini, Nithyapriya, Sasidharan, Formulation and evaluation of ciprofloxacin dental films for periodontitis, Journal of Chemical and Pharmaceutical Research, 4(6) (2012) 2964-2971.
15. Sharon Furtado, SrinivasanBharath, BasappaVeerbadraiahBasavaraj, Sindhu Abraham, RajamanickamDeveswaran, VaradharajanMadhavan, Development of chitosan based bioadhesivebilayered patches of metoprololtartarate, International journal of pharmaceutical sciences review and research, 4(3) (2010) 198-202.
16. S. Singh, S. Jain, M. S. Muthu, S. Tiwari, and R. Tilak, Preparation and Evaluation of Buccal Bioadhesive Films Containing Clotrimazole, American Association of Pharmaceutical Scientists. 9(2) (2008) 660-667.