ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

TRIPLE-LAYERED BUCCAL FILMS: DEVELOPMENT AND EVALUATION FOR HYPERTENSION CONTROL

Srivastava N*, Thakur S, Kumar R

School of Pharmaceutical Sciences, RIMT University, Mandi Gobindgarh, Punjab
PCTE Group of Institutes, Baddowal Cantt, Faculty of Pharmaceutical Sciences, Ludhiana

*Corresponding author E- mail: **nehasrvstva@gmail.com**

ARTICLE INFO

Key words:

Buccal adhesive film, Atenolol, Nifedipine, triple layer

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Nifedipine and atenolol were combined to create a three-layer buccal film that effectively treated hypertension with fewer adverse effects. A triple layer was created, with one layer containing atenolol in an immediate release layer separated by the backing layer, and one layer containing nifedipine in a prolonged release layer to sustain the release rate and avoid first-pass metabolism. The materials used for preparing the film include hydroxyl propyl methyl cellulose (HPMC E15), chitosan, polyvinyl chloride (PVC), and polyvinyl pyrrolidone K-30 (PVP K-30). Several physiochemical parameters, including folding endurance, thickness of the film (mm), weight variation, surface pH, texture, and tensile strength, were assessed for the produced formulation. The *in vitro* bioadhesive strength, swelling index, and *in vitro* drug release studies were studied. The study showed that the film has good physical parameters and *in vitro* release showed 91.63 % release for immediate release and 96.91 % release for sustained release layer. The *in vitro* bioadhesion studies were carried out using goat mucosa suggesting good bioadhesion of the films for 24 h. Thus, from the study, it can be concluded that the triple-layer film was prepared and evaluated which can be used for effective control of hypertension.

INTRODUCTION:

The conventional delivery system is widely accepted system across the globe and has traditionally followed for years. However conventional therapy has several disadvantages such as adverse effects, inefficiency in reaching the target site, failure to adhere, frequent dosing, and many more. The problem related to the traditional dosage form can be surpassed by alternative and novel delivery systems which are blooming up leading to various new paths in the science of delivery systems.[1,2] The efficiency of the drug can be improved by increasing the drug localization time at the site of absorption bypassing different metabolic processes.[3] Among the different methods of drug delivery, the buccal delivery system is one of the prominent methods of drug delivery which can overcome many conventional drawbacks. Due to its abundance of blood capillaries, the buccal mucosa is a great place to provide medication. This method of administration allows the drugs to enter the bloodstream directly, avoiding the stomach environment and first-pass metabolism, thus improving the drug's bioavailability.[4] Because self-application is possible, the buccal delivery system is safe and has good patient compliance. In an emergency case, it is

possible to stop the distribution system at any time. When compared to alternative distribution methods or buccal tablets, the buccal film is more flexible and palatable.[5,6] Nifedipine is a cardiovascular medication that is a member of the calcium channel blocker class. It is a preferred medication for the management of angina pectoris and hypertension. Nifedipine possesses a shorter biological half-life of two hours and a rapid hepatic metabolism. Nifedipine is a good option for the extended layer of triple-layer film because of its low absorption and low therapeutic dose of 20 mg.[7,8] Atenolol is a selective antagonist that falls within the class of β-receptor blockers, atenolol is used to treat heart failure, angina, hypertension, and myocardial infarction. Its half-life is 6-7 hours, and its first-pass metabolism is quite low (>10%). This medication does not significantly affect hepatic metabolism when taken orally.[9,10] In the present work, Atenolol is incorporated in the immediate (fast) release layer and nifedipine in the controlled release layer. This combination of Atenolol and nifedipine is more effective in the control of hypertension than atenolol alone. As this combination is more effective in lowering blood pressure at a low dose than atenolol alone. So, lowering the dose of atenolol can decrease the side effects of this drug without reducing the therapeutic effect. The immediate release layer was formulated with HPMC (E-15) polymer, and the sustained release layer was formulated with Chitosan, PVA, and PVP K-30. Both the layers were strongly attached with the help of PVA.

MATERIALS AND METHOD

Materials

Nifedipine and Atenolol were sample drugs gifted by Mylan Laboratories Limited, Hyderabad. Chitosan was purchased from a commercial source Hi-Media, PVA, and PVP were purchased from Qualikems Fine Chem Pvt. Ltd. HPMC was purchased from Central Drug House (P) Ltd. All the reagents and chemicals used are of analytical grade; double-distilled water was used for the research purpose.

Methods

Compatibility studies: Interaction between the drug and the excipients was determined by using FTIR spectrophotometry. KBr pellet technique was used for FTIR studies. FTIR spectrum of plain drug Nifedipine and Atenolol, plain polymers, and a mixture of polymers and the drug were performed.[11]

Preparation of Triple-layered buccal films: All three layers of the buccal film were made up of different polymers and their combination to provide different drug release rates. These films were fabricated by solvent casting technique using a petri plate. Propylene glycol was incorporated to increase the plasticity and tween 60 was added as permeation enhancer. [12] These films were cast in three steps:

Preparation of immediate release layer: Accurately weighed quantity of HPMC was solubilized in 10ml of double distilled water and with rapid and consistent stirring propylene glycol was gradually added. The drug was added and mixed uniformly. After mixing, the solution was kept for sonication to make the solution clear and bubble-free. Then the resultant mixture was transferred carefully into the petri plate. The film was dried at 50ºC for 3 hours in a hot air oven.[12] The formulation details of the immediate layer are given in Table 1.

Preparation of backing layer: 10 ml of 10% PVA solution was prepared by solubilizing PVA in water at 60 ºC then poured on the dried HPMC layer and again

drying was carried out at 60 ºC for 1 hour in the oven.[13]

Preparation of sustained release layer

An accurately weighed quantity of chitosan was taken and mixed with 100 ml of acetic solution (1.5 % v/v) and stirred continuously till it was completely dissolved.[14] 20 ml of chitosan solution was taken and PVP and PVA were dissolved properly. The drug was mixed properly with tween 60 and propylene glycol (plasticizer) using a magnetic stirrer under constant stirring, then the chitosan solution was mixed with the drug. All the components were mixed well. The resultant mixture was kept aside without disturbing the air bubbles which gradually disappeared. The resultant preparation was placed on the already-dried film of HPMC and PVA and dried at 50 ºC for five hours in tray dryers. After drying the resultant film was cut into 2.5cm^2 sizes for evaluation studies.[15] The formulation details of the sustained release layer are given in Table 2. For easy evaluation studies, the films were prepared separately (Immediate release layer + backing layer) and (backing layer + Sustained release layer). These films were made by following only two steps mentioned above. After evaluation, the films with the best results were cast in triple-layer films, and their physical parameters were evaluated.

Evaluation of buccal film: For the easy evaluation of triple-layer films two separate films are prepared. One with immediate release film attached to the PVA Backing layer and the second with sustained release film attached to the PVA backing layer. After optimization, the films with better results are combined finally to produce the triple layer films. Evaluation of each layer was performed individually.

Physical Appearance: Physical appearance is done by regular inspection by visualization and surface texture evaluation by feel or touch.[16]

Uniformity of weight of films: Weigh six films individually using digital balance and average weights were calculated.[17]

Thickness of films: Three randomly selected films were used for thickness measurement. The thickness of the film was determined by using a venire caliper with a least count of 0.01 mm at various spots and an average was noted.[17]

Folding endurance: Folding endurance is quantitatively measuring the flexibility of films. The folding endurance of films was measured by folding films repetitively at a similar position until the film broke. The number of times that a film can be folded in a comparable manner without breaking indicates how durable the prepared film is for folding.[18, 19]

Swelling index: The swelling index was measured by soaking pre-weighed film in approx. 50 ml of water. Carefully take out the film from the water after 8 hours and it is blotted with the help of filter paper and weighed accurately.[20] The swelling index is calculated by,

$$\text{Percent Swelling Index} = \frac{\text{Wet weight - Dry weight}}{\text{Dry weight}} \times 100$$

Surface pH of films: The pH of the film surface was determined by placing the electrode on the film surface and equilibrating it for 1 min. Three readings were noted, and the mean was calculated.[21]

Tensile strength: Tensile strength is an important characteristic for the buccal film to maintain its integrity at the time of administration. To split the film into two

pieces, force or applied stress is needed. The device is made in a lab to ascertain the buccal film's tensile strength, as depicted in Figure 1. The weighing balance was modified and employed to determine the strength of the prepared film. In the weighing balance, one side of the pan is replaced with a plate and it is tied with a hook to hold the film in place. The balance is equilibrated by placing weight on the right hand of the balance. Balance was changed so that it can fix the film in between 2 hooks holds on horizontal beams. On one side the film was attached and on the other pan weights were added.[22] Tensile Strength is calculated as

$$T = M \times g / b \text{ xt Dynes/cm}^2$$

Where, T is the stress to break a film, M is the mass added to tear the film, g is the acceleration due to gravity, is the breadth of the film (cm), and t is the thickness of the film (cm).

Determination of drug content: The whole film was taken and cut into equal pieces of 2.5 cm^2 length. For drug content determination, a piece of film was placed in a mortar and crushed with the help of a pestle. Methanol was added to the crumbled film, evenly triturated to dissolve the drug component, and then further diluted to a volume of 100 milliliters. Using Whatman filter paper, the final solution was filtered. Using a UV spectrophotometer (UV-1700 Double beam, SHIMADZU) set at 238 nm for nifedipine and 226 nm for atenolol, the drug content is measured.[23]

$$\% \text{ drug loading} = \text{Practical loading of drug in the film/ Theoratical drugloading } X\,100$$

***In-vitro* drug release study:** For the *in-vitro* release study, the USP dissolution apparatus was used. The dissolution was carried out using 400 ml of phosphate buffer pH 6.8 at 100 rpm using apparatus type II. A piece of film (2.5 cm^2) was used and stuck to a glass slide to avoid floating the film above the media. At different time points, 2 ml samples were taken and placed with a buffer medium so that the sink condition was maintained. The absorbance of the sample was measured at 226 nm and cumulative release was determined.[24]

Mucoadhesion study: A mucoadhesion study was implemented for the sustained release layer made up of chitosan and nifedipine drugs. This study was performed with the help of a modified physical balance technique as shown in Figure 2. Bioadhesive performance is based on the stress applied to remove the film from the membrane surface. Modified physical balance is utilized to determine the mucoadhesive property. Balance with two arms is used for the study, the right pan is removed and replaced with a glass slide to which a film of 2.5 cm^2 has been attached at the center. On the left pan, a beaker is placed to counterbalance the right arm. The burette is placed above the beaker at a height so that water can be added directly to the beaker. A glass beaker is placed below the glass slide containing the film. A beaker is filled with pH 6.8 phosphate buffer to mimic saliva condition. A beaker is placed on a magnetic stirrer with a temperature controller to maintain the temperature at 37±0.5 ºC. 3 cm of goat mucosa is isolated and placed tightly on another glass slide and this slide is placed below the other glass slide carrying the formulated film. The film was moistened using buffer pH 6.8 and it is kept in phosphate buffer pH 6.8 for 30 sec for swelling and hydration. The glass slide containing the film is placed in contact with another glass slide containing mucosa. The assembly was kept as such for 3 min to create adhesive force between the force and tied mucosa. After 3 minutes, water is added to the beaker with the help of a burette. The

water was added continuously until the film detached completely from the membrane surface. The weight of water collected at the time of detachment is noted. The water's weight required to remove the film is used to determine the mucoadhesive force.[24, 25]

$$\text{Mucoadhesion force, } F = \frac{(W_w \times g)}{SA}$$

W_w= weight of water (g)
g =Acceleration due to gravity (cm/s^2)
SA = Surface area of the film (cm^2)

Permeability study: Permeation study was done with the help of dialysis membrane using Frank diffusion cell. In this diffusion cell, there is one receptor compartment and one donor compartment separated by a dialysis membrane. The receptor compartment contains 50 ml phosphate buffer (pH 6.8) plus 0.5% w/v tween 60. The film was placed on the dialysis membrane between the donor and recipient compartments. To maintain a temperature of 37 ± 0.5 ˚C, the assembly is put on a magnetic stirrer equipped with a temperature controller. The stirring was done at 50 rpm. At regular intervals, 5 ml of the sample was removed and replaced with the same volume of phosphate buffer that was intended. Using a UV spectrophotometer (Shimadzu, Japan), the drug release was measured at 238 nm.[26]

Drug release kinetic study: Drug release kinetics was determined to determine the release rate of the film and also to treat it with mathematical models to determine the pattern of release such as zero order, first order, Higuchi and Peppas equations.[27]

RESULTS AND DISCUSSION

Drug–Polymer Compatibility:

IR Spectra of Nifedipine and Polymers: IR spectra were determined for Nifedine individually and in combination with polymers. An IR spectrum of nifedipine indicates the peaks 3330.60 cm^{-1} which is the region of N-H, str, 2962.76 cm^{-1} corresponds to C=C str, 1529.39 cm^{-1} corresponds to Ar-NO str and 1678.98 cm^{-1} corresponds to C=O str.[28] These are the characterized peak of the drug nifedipine and the same peaks were present in the IR spectra of nifedipine with the polymers as shown in Figures 3 and 4.

IR Spectra of Atenolol and Polymers: In the IR spectra of the drug Atenolol, the peak indicates characteristic peaks at 3354.55 cm^{-1} of N-H stretching and 1636.52 cm-1 of C=O stretching of the amide group. The peaks at 1417.62 cm^{-1} and 1242.80 cm^{-1} correspond to the presence of the alcoholic –OH group. The presence of a peak at 3358.55 cm^{-1} is of N-H stretching and 1636.88 cm^{-1} corresponds to C=O stretching of the amide group respectively. The peaks present at 1419.51 cm^{-1} and 1241.16 cm^{-1} show the presence of the alcoholic –OH group. All the prominent peaks of the drug were unchanged in the mixture of drug and polymer. The spectrum indicated that there is no drug excipient interactions.[29] Figure 5 and 6 represents the spectrum of atenolol and physical mixture of atenolol and the polymers. ATN1 formulation is found to have less flexibility due to higher concentration of polymer in the prepared films, this formulation also has very hard texture. As the concentration of polymers is decreased to drug-polymer ratio 1:1 the formulation is found to have increased flexibility. ATN3 formulation is found to have flexible texture as compared to the other formulations having folding endurance of 178 folds as well as comparatively good tensile strength. In case of formulation ATN4 and ATN5 the concentration of polymer is constantly decreasing which causes brittleness of the formulation due to high drug content. Different physical

parameters are tabulated in Table 3. The percent cumulative drug release is shown in Figure 7. Cumulative % drug release was found to be less with the formulation ATN1 and ATN2 with drug release of 69.94% and 82.02% respectively in 30 minutes time interval. But in case of ATN 3 and ATN 4 it was increased to 91.63% and 94.01% respectively. It was due to decrease in polymer concentration, which results in increased drug release. In case of ATN3 68.18% of drug release was found after 10 minutes. The required drug release was above 90% within 30 minutes. This criterion was passed by ATN3, ATN4 and ATN5 formulation. But ATN4 and ATN5 formulation failed the physical parameter test as they have less folding endurance and brittle texture. So ATN3 is found best among all these formulations. Drug content study indicates the uniformity of drug content in the prepared formulation. Table 4 represents the drug content studies of immediate release layer. Percentage of drug found to be 95.01%, 98.47%, 98.26%, 96.38%, 95.23% in formulation ATN1, ATN2, ATN3, ATN4, ATN5 respectively which is satisfactory in all formulations. Hence the formulation ATN3 had passed all the parameters having good tensile strength, folding endurance as well as percentage drug release of 91.63% in 30 minutes and percentage of drug content 98.26%. So ATN3 formulation was the best one among all the immediate release formulations. Physical Parameters tests for sustained release layer was tabulated in Table 5. Formulation from NPN1 to NPN9 was found to be flexible and has good tensile strength, which ensures that the films can be easily folded without breakage. Folding endurance of the film was found to be more than 200 folds considered as good folding endurance of film. Surface pH should be near neutral and for the prepared film pH is in the range of 5.6 to 6.3 which was in satisfactory range. All the films have good tensile strength due to the backing layer of PVA. Weight of films increases according to batches due to increase in polymer amount in each batch. Percentage drug release in the duration of 8 hrs was 61.4, 70.23, 75.83, 79.73, 80.31, 82.26, 84.02, 86.45 and 89.73 respectively for formulation NPN1 to NPN9. NPN1 composed of only chitosan produces very low drug release within 8 hr. Slowly the increase in drug release was found by adding PVP in NPN2 and further increasing its concentration in NPN3, NPN4, NPN5 to 0.4,0.5,0.6% respectively. The presence of the hydrophilic additive, PVP in chitosan films seemed to increase the surface wettability and swelling of the films. When PVA is added along with PVP, it was observed that drug release was further improved.[30] In the formulation NPN7, NPN8, NPN9 the drug release was found to be 84.02, 86.45 and 89.73 respectively which is in satisfactory range. As compared to all formulations NPN9 has the maximum drug release within 8 hrs. *In-vitro* permeability data was represented in Figure 8.

Swelling studies: Swelling index affects the release rate of the drug from the formulation. Table 6 represents the percentage swelling index. It was observed that with the increment in the amount of PVP from 0.3% to 0.6% increases the swelling index from 14.69% to 20.99%. It was further increased by adding PVP and PVA to the NPN9 formulation to 27.22%. Swelling affects the drug release form the formulation. With increased swelling the drug release also increased. Swelling was amplified with the increment in the concentration of PVP and PVA in the prepared formulations.[31, 32] Table 7 represents the drug content and mucoadhesion studies of sustained release layer. Bioadhesive strength was found to be increased, by the addition of PVP and PVA in the formulations. PVA polymer produced good bioadhesive properties when it was

used in addition with PVP.[32] According to literature chitosan possess good bioadhesive strength in absence of drug, but after the addition of drug its bioadhesive strength decreases. Decrease in the bioadhesive strength depends on the amount of drug added. Other polymers like PVA and PVP are used to increase bioadhesive strength and swelling properties.[33] According to the results NPN9 formulation was considered the best formulation having high release rate of 89.73% and good bioadhesive strength among all formulations.

Table 1: Formulation details of Immediate Release Layer

FC	Drug (mg)	HPMC E-15 (mg)	Plasticizer (%)	Distilled water (ml)
ATN1	50	150	5%	10
ATN2	50	100	5%	10
ATN3	50	50	5%	10
ATN4	50	25	5%	10
ATN5	50	12.5	5%	10

FC = Formulation code

Table 2: Formulation details of Sustained release Layer

FC	Drug (mg)	Chitosan (1.5%v/v acetic acid)	PVP K-30 (%)	PVA (%)	Tween60 (%)	Propylene Glycol (%)
NPN1	20	1%	-	-	1%	5%
NPN2	20	1%	0.3%	-	1%	5%
NPN3	20	1%	0.4%	-	1%	5%
NPN4	20	1%	0.5%	-	1%	5%
NPN5	20	1%	0.6%	-	1%	5%
NPN6	20	1%	0.6%	0.3%	1%	5%
NPN7	20	1%	0.6%	0.4%	1%	5%
NPN8	20	1%	0.6%	0.5%	1%	5%
NPN9	20	1%	0.6%	0.6%	1%	5%

*FC = Formulation code

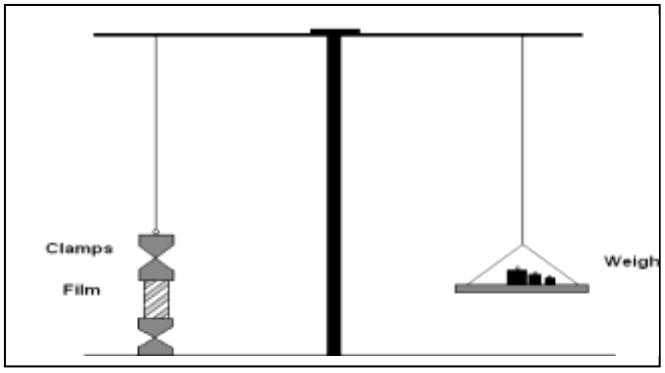

Figure 1. Modified tensile strength tester

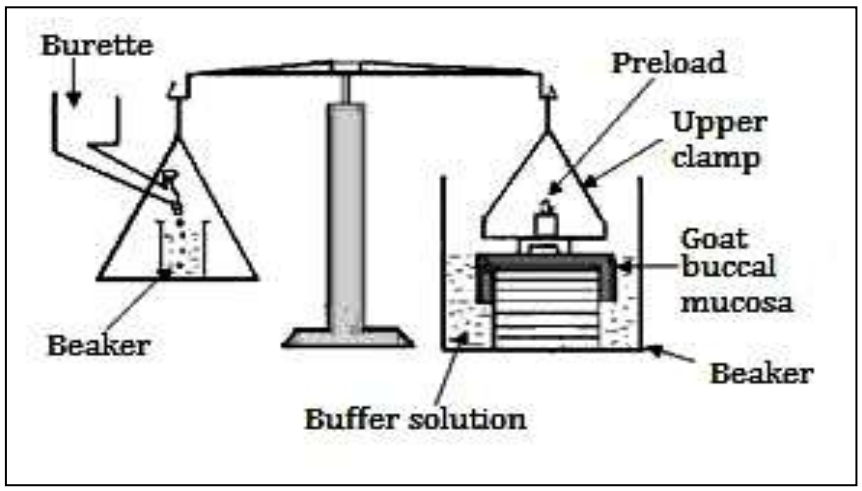

Figure 2. Physically modified balance for the measurement of Mucoadhesive strength

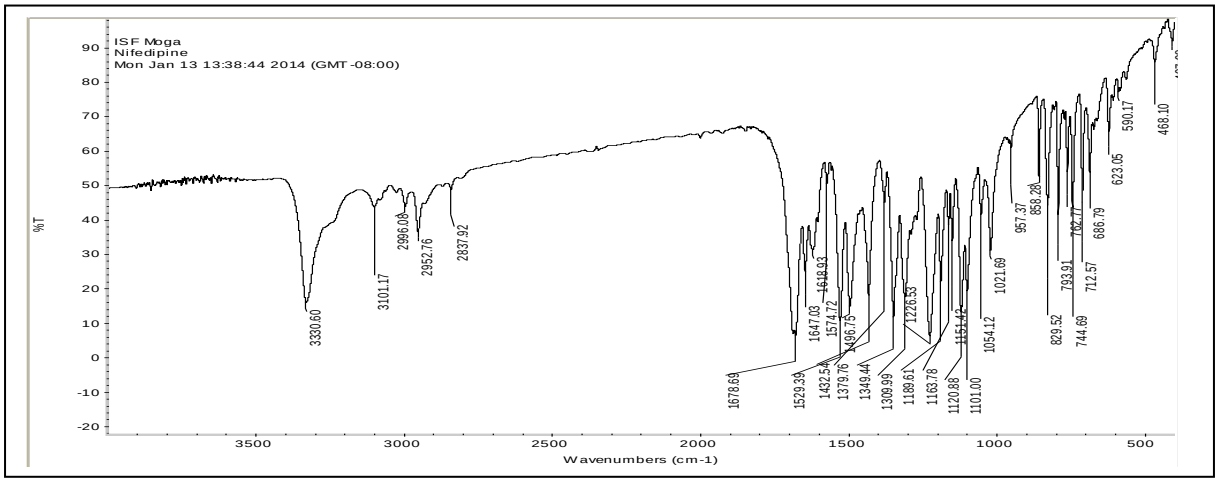

Figure 3-IR spectra of nifedipine

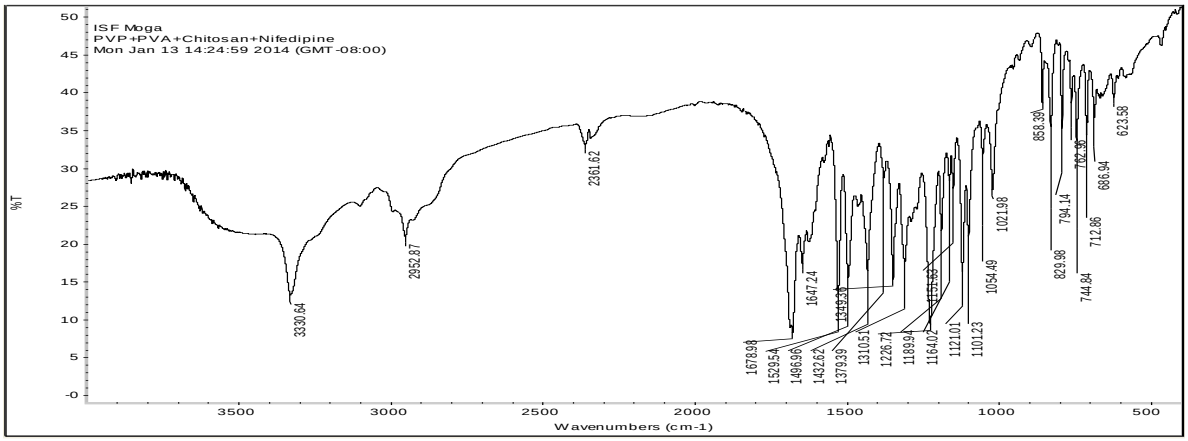

Figure.4-IR spectra of Nifedipine, PVP, PVA, Chitosan

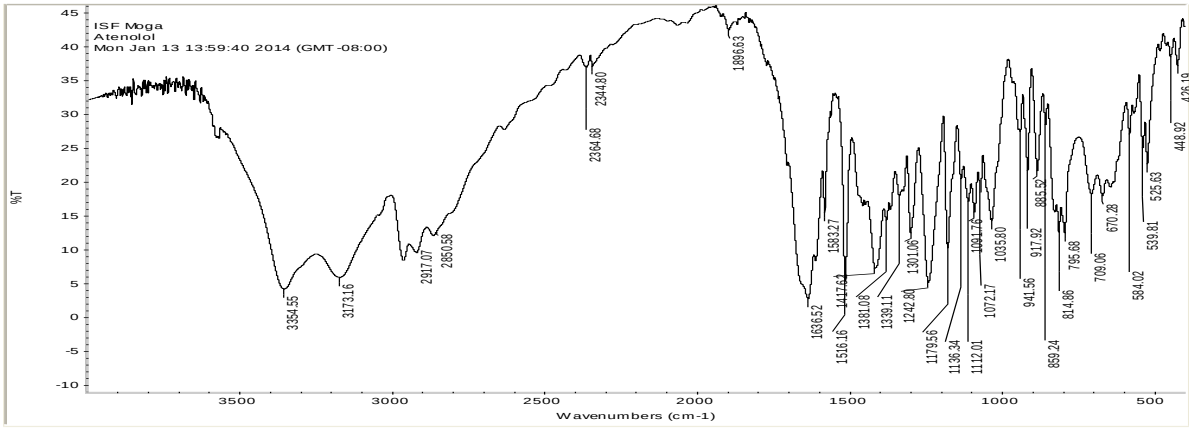

Fig.5-FTIR Spectrum of Atenolol

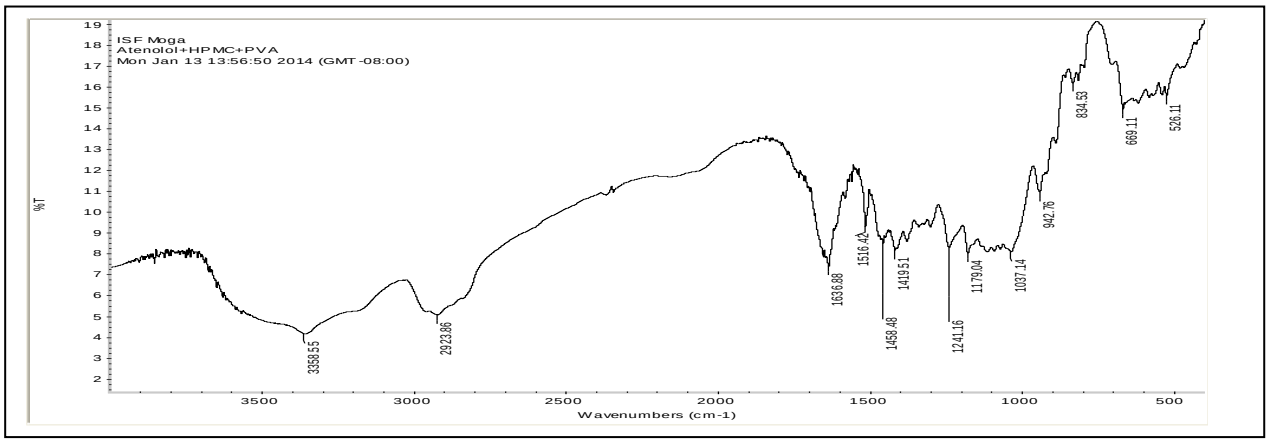

Fig.6-FTIR Spectrum of Atenolol, HPMC, PVA

Evaluation results of immediate release layer (atenolol)

Table 3: Physical parameters test results for immediate release film

Formulation code	Surface pH	Folding endurance	*Thickness (mm)	Texture	*Weight variation (mg)	Tensile strength (dynes\cm^2)
ATN1	7.1 ±0.01	145	0.53 ± 0.05	Less flexible	203 ± 5.7	9.2×10^6
ATN2	7.22 ±0.03	156	0.56 ± 0.05	Flexible	176 ± 5.7	8.7×10^6
ATN3	0.63 ± 0.05	178		Flexible	110 ± 5.7	7.7×10^6
ATN4	0.66 ± 0.05	60		Brittle	93.3 ± 5.7	7.4×10^6
ATN5	0.66 ± 0.05	30		Brittle	83.3 ± 5.7	7.4×10^6

*Mean ± SD, n=3

Table 4: Drug content study for immediate release layer

Formulation code	ATN1	ATN2	ATN3	ATN4	ATN5
*Drug content %	95.01 ± 3.83	98.47 ± 2.70	98.26 ± 1.47	96.38 ± 3.60	95.23 ± 2.53

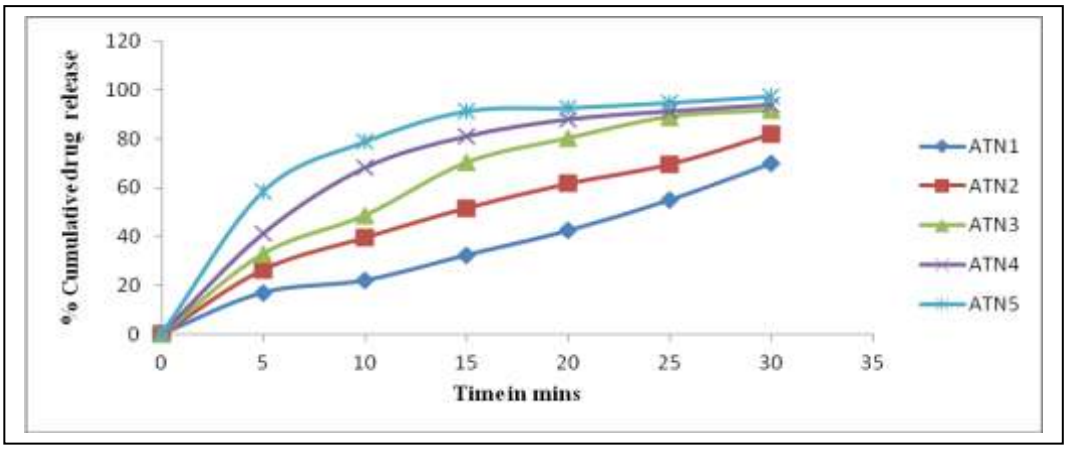

Figure 7. Percentage cumulative drug release for Immediate release layer *Mean ± SD, n=3

Evaluation results of sustained release layer (nifedipine)

Table 5: Physical Parameters tests for sustained release layer

Formulation code	*Surface pH	Folding endurance	*Thickness (mm)	Texture	*Thickness (mm)	*Weight variation (mg)	Tensile strength (dynes/cm^2)
NPN1	5.9 ± 0.08	>200	0.43±0.05	Flexible	0.43±0.05	83.3 ± 5.7	6.2 × 10^6
NPN2	5.6 ± 0.12	>200	0.46±0.05	Flexible	0.46±0.05	90 ± 8.1	6.6 × 10^6
NPN3	6.3 ± 0.02	>200	0.43±0.05	Flexible	0.43±0.05	103.3 ± 5.7	7.3 × 10^6
NPN4	6.1 ± 0.05	>200	0.46±0.05	Flexible	0.46±0.05	106.6 ± 5.7	8.6 × 10^6
NPN5	5.8 ± 0.04	>200	0.46±0.05	Flexible	0.46±0.05	113.3 ± 5.7	8.6 × 10^6
NPN6	6.2 ± 0.02	>200	0.53±0.05	Flexible	0.53±0.05	116.6 ± 5.7	9.2 × 10^6
NPN7	6.0 ± 0.01	>200	0.56±0.05	Flexible	0.56±0.05	123.3 ± 5.7	9.7 × 10^6
NPN8	6.2 ± 0.03	>200	0.56±0.05	Flexible	0.56±0.05	126.6 ± 5.7	9.7 × 10^6
NPN9	6.3 ± 0.02	>200	0.63±0.05		0.63±0.05	136.6 ± 5.7	9.8 × 10^6

*Mean ± SD, n=3

Table 6: Percentage swelling index

Formulation code	NPN 1	NPN 2	NPN 3	NPN 4	NPN 5	NPN 6	NPN 7	NPN 8	NPN 9
Swelling index	12.27 ± 0.27	14.69 ± 2.30	15.42 ± 1.34	19.06 ± 2.20	20.99 ± 2.81	24.38 ± 3.34	24.13 ± 2.32	26.49 ± 2.66	27.22 ± 1.21

Table 7: Drug content and Mucoadhesion studies

Formulation code	Drug content %	Mucoadhesion (g)	Bioadhesive force (N)	Bond Strength (N m^{-2})
NPN1	95.67 ± 2.5	3.2 ± 1.2	0.031	124
NPN2	93.22 ± 2.0	6.51 ± 2.3	0.063	252
NPN3	91.35 ± 3.9	8.4 ± 1.5	0.082	328
NPN4	97.92 ± 1.0	9.7 ± 2.1	0.095	380
NPN5	99.09 ± 3.3	11.3 ± 2.6	0.110	440
NPN6	95.71 ± 2.2	16.4 ± 2.5	0.160	640
NPN7	92.03 ± 3.1	18.2 ± 1.9	0.178	712
NPN8	97.33 ± 3.2	19.4 ± 2.2	0.190	760
NPN9	96.91 ± 1.2	22.2 ± 2.1	0.217	868

Table 8: Kinetic data of nifedipine mucoadhesive buccal film

FC	First-order (R^2)	Zero-order (R^2)	Higuchi (R^2)	Peppas plot	
				(R^2)	n-value
NPN1	0.9949	0.9949	0.9387	0.9883	0.8763
NPN2	0.9552	0.9551	0.9789	0.9673	0.5486
NPN3	0.9002	0.9001	0.9978	0.9953	0.4957
NPN4	0.8750	0.8749	0.9970	0.9936	0.4595
NPN5	0.8470	0.8469	0.9961	0.9949	0.4426
NPN6	0.8898	0.8897	0.9953	0.9837	0.4580
NPN7	0.8649	0.8648	0.9959	0.9876	0.4372
NPN8	0.8415	0.8414	0.9966	0.9948	0.4247
NPN9	0.8219	0.8217	0.9957	0.9995	0.4215

Table 9: Physical Parameter tests for triple layered optimized film

Formulation code	Texture	Surface pH	Folding endurance	Thickness(mm)
TL1	Flexible	6.8 ± 0.02	168	0.93±0.05

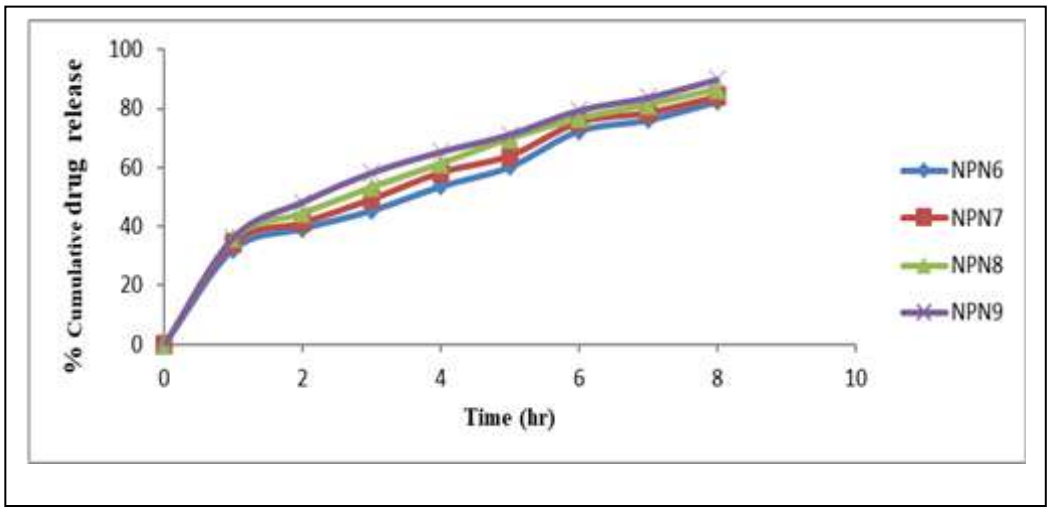

Figure 8 (a). *In-vitro* permeability studies (NPN1-NPN5)

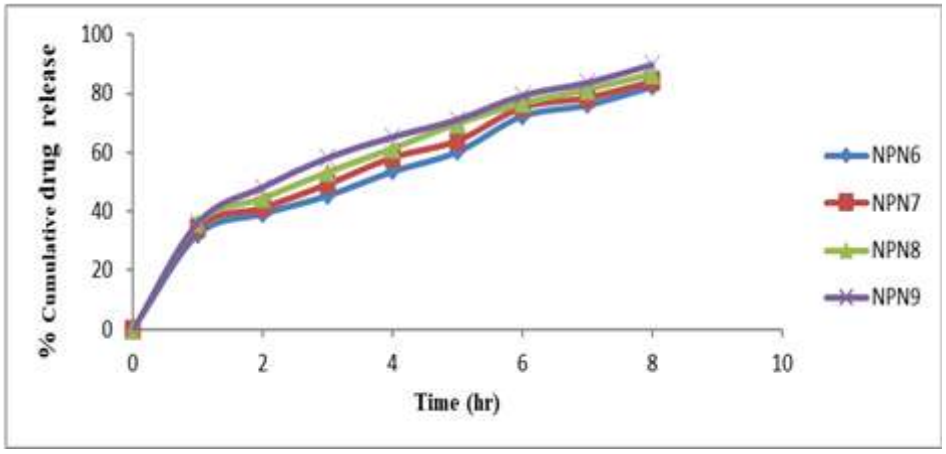

Figure 8 (b). *In-vitro* permeability studies (NPN6 – NPN9

All the formulations were found to have acceptable tensile strength as well as folding endurance due to application of PVA backing layer during preparation. Swelling index of NPN9 formulation was found to be good which effects the release rate of the drug through formulation. ATN3 and NPN9 formulations were found to be optimized and show best results. So their polymer concentrations were used in the manufacturing of triple layer buccal films contains atenolol and nifedipine drugs in two different layers.

Drug Release Kinetics: The drug release mechanism can be tracked using different kinetic models. Zero order, First order and Higuchi models while Korsmeyer-Peppas model are used to study different release pattern. The results are summarized in Table 10. The dissolution data of different batches of film is subjected to different models and with the help of regression value best fitted one is selected. It is observed that if value for R^2 is higher, higuchi model is better fit as compared to other. Higuchi model tells the release pattern as diffusion process related to fick's law.[33] Korsmeyer-Peppas release model used to study the most favorable mechanism for the drug to be released from delivery system. This model indicates more than one type of release mechanism and helps to determine the release from polymeric dosage form. The graph is plotted against log cumulative percent release and log time, and the release rate constant, k, and release exponent, n, are calculated. According to the literature, $0.45 \leq n$ implies fickian diffusion, $0.45 < n < 0.89$ indicates non-fickian transport, $n = 0.89$ indicates relaxational transport, and $n > 0.89$ indicates supercase II transport.[34,35] Table 8 represents kinetic data of nifedipine mucoadhesive buccal film. the The slope value (n) was calculated for peppas exponential plot for NPN9 formulation, which suggests that drug released by fickian diffusion mechanism. The slope value (n) of formulation NPN1 was 0.8763 which determines the drug release occurs by non-fickian transport mechanism. From formulation NPN1 to NPN9 the drug transport mechanism changes from non-fickian to fickian diffusion due to the addition of hydrophilic polymers PVP and PVA, which means the hydrophilic character of formulation increases from NPN1 to NPN9. It helps in increased swelling and increased drug release.

Physical Parameter tests for triple layered film: Table 13 represents physical parameter tests for triple layered optimized films. Physical parameters tests were done to ensure the texture of the triple layer film. Texture was found to be flexible, and had folding endurance of 168 folds which is satisfactory and surface pH was 6.8. This formulation was free from any kind of interaction between polymers and the drug. Drug release characters for this film have been already optimized and confirmed by making separate double layer films. Mucoadhesion have also been confirmed and found satisfactory. This was the final formulation designed to provide the desired characters having immediate plus sustained drug release and bypass the hepatic first pass metabolism of nifedipine.

CONCLUSION

Nifedipine and Atenolol are the first-line drugs for treating high blood pressure (BP) and angina pectoris. A low-dose combination of the two groups of medications helps to regulate hypertension without changing the patients' adrenergic and hormonal condition. Low does combination can also prevent dose related side effects. Due to sustained release formulation the duration of action is increased and by preventing hepatic first pass metabolism, bioavailability of drug get increased. Hepatic metabolism is prevented by changing the route of drug administration. Flexibility in physical state, shape, size and surface increase the comfort level of patient due to ease of administration and termination. Triple layer formulation is very helpful in providing dual release pattern following immediate release of atenolol to sustained release of nifedipine. By combining these two optimized formulations the final triple layer film is made having flexible texture, good folding endurance, and required release rate from the formulation. From the result, it is conferred that the drug is distributed uniformly within the film. Chitosan helps in film forming and also exhibits good mucoadhesion properties. The drug release rate is improved by addition of PVP in the chitosan base matrix system. As a result, Chitosan with PVP and PVA can match the optimum parameters for buccal devices, potentially bypassing substantial hepatic first pass metabolism and increasing bioavailability.

Acknowledgement: We are sincerely thankful to Mr. Vipin Bongade for provinging the gift sample of Nefidipine from Matrix laboratories limited, Hyderabad.

Conflict of interest: No conflict of interest.

REFERENCES:

1. Adepu, Shivakalyani, and Seeram Ramakrishna. Controlled drug delivery systems: current status and future directions. Molecules, 26.19 (2021) 5905, 1-45.
2. Ezike, Tobechukwu Christian, et al. Advances in drug delivery systems, challenges and future directions. Heliyon 9, 6 (2023) 1-17.
3. Alqahtani, Mohammed S., Mohsin Kazi, Mohammad A. Alsenaidy, and Muhammad Z. Ahmad. Advances in oral drug delivery. Frontiers in pharmacology 12 (2021), 618411, 1-21.
4. Jacob, Shery, Anroop B. Nair, Sai HS Boddu, Bapi Gorain, Nagaraja Sreeharsha, and Jigar Shah. "An updated overview of the emerging role of patch and film-based buccal delivery systems. Pharmaceutics 13, no. 8 (2021): 1206 1-39.
5. Macedo, Ana S., Pedro M. Castro, Luís Roque, Natália G. Thomé, Catarina P. Reis, Manuela E. Pintado, and Pedro Fonte. "Novel and

revisited approaches in nanoparticle systems for buccal drug delivery. Journal of Controlled Release 320 (2020): 125-141.
6. Gupta, Sabnam, Sudip Das, Abhay Singh, and Suman Ghosh. A Brief Review on Bucco-adhesive Drug Delivery System. Journal of Drug Delivery and Therapeutics 11, no. 4-S (2021): 231-235.
7. Narmetova, S. Y., and J. S. Mardiyeva. The Advantage of the Drug Nifedipine in the Treatment of Arterial Hypertension. American Journal of Language, Literacy and Learning in STEM Education (2993-2769) 1, no. 6 (2023): 280-282.
8. Nifedipine Uses, Dosage & Side Effects - Drugs.com, Feb 2024.
9. Sharma, R. A. C. H. A. N. A., and Uma Sharma. Formulation and characterization of atenolol-loaded gellan gum nanoparticles. Indian J. Pharm. Sci 83 (2021): 60-65.
10. Atenolol: Uses, Interactions, Mechanism of Action | DrugBank Online, Feb
11. Omari, Derar M., Yazan Akkam, and Assayed Sallam. Drug-excipient interactions: an overview on mechanisms and effects on drug stability and bioavailability. Annals of the Romanian Society for Cell Biology (2021): 8402-8429.
12. Jose J, Sisira MV, Prasanth ML, Prasanth CS, Pradeep PS. Design and Characterization of Buccal Films of Benzocaine for Mouth Ulcer. Polymer. 2021;10(250):250- 265.
13. Nair, Anroop B., Jigar Shah, Shery Jacob, Bandar E. Al-Dhubiab, Vimal Patel, Nagaraja Sreeharsha, and Pottathil Shinu. Development of mucoadhesive buccal film for rizatriptan: In vitro and in vivo evaluation. Pharmaceutics 13, no. 5 (2021): 728 1-16.
14. Korelc K Korelc, Karin, Bjarke Strøm Larsen, Mirjana Gašperlin, and Ingunn Tho. Water-soluble chitosan eases development of mucoadhesive buccal films and wafers for children. International journal of pharmaceutics 631 (2023): 122544 1-13.
15. Korelc K, Larsen BS, Gašperlin M, Tho I. Water-soluble chitosan eases development of mucoadhesive buccal films and wafers for children. International journal of pharmaceutics. 2023 Jan 25; 631:122544. 1-13
16. Hassan, Alharith AA, Katalin Kristó, Yousif H-EY Ibrahim, Géza Regdon Jr, and Tamás Sovány. Quality by Design-Guided Systematic Development and Optimization of Mucoadhesive Buccal Films. Pharmaceutics 15, no. 10 (2023): 2375 1-24.
17. Kelemen, András, Effects of sucrose palmitate on the physico-chemical and mucoadhesive properties of buccal films. Molecules 25, no. 22 (2020): 5248 1-14.
18. Thella, Ashok Babu and K. Thejomoorthy. Formulation Development And In Vitro Characterization of Atorvastatin Buccal Patches. International Journal of Pharmacognosy and Chemistry (2020): 1-11.
19. Zamora, Cristina M. Pérez, Ariel G. Michaluk, Diego A. Chiappetta, and María B. Nuñez. Herbal buccal films with in vitro antibacterial and anti-inflammatory effects. Journal of Herbal Medicine 31 (2022): 100527.
20. Suksaeree J, Chaichawawut B, Srichan M, Tanaboonsuthi N, Monton C, Maneewattanapinyo P, Pichayakorn W. Applying design of experiments (DoE) on the properties of buccal film for

nicotine delivery. e-Polymers. 2021 Aug 6;21(1):566-74.
21. Kunal, V. Bhambar, S. Bhambar Rajendra, B. Darekar Avinash, P. Gadakh Pravin, and Aher Rutuja. Formulation and Characterization of Buccal Patches of Oxaceprol. Research Journal of Pharmacy and Technology 15, no. 12 (2022): 5512-5516.
22. Rohani Shirvan, Anahita, Nahid Hemmatinejad, S. Hajir Bahrami, and Azadeh Bashari. Fabrication of multifunctional mucoadhesive buccal patch for drug delivery applications. Journal of Biomedical Materials Research Part A 109, no. 12 (2021): 2640, 1-17.
23. Mann, Garima, Pramod M. Gurave, Ankur Kaul, K. Ganesh Kadiyala, Manisha Pokhriyal, Rajiv K. Srivastava, Arun Kumar, and Anupama Datta. Polymeric and electrospun patches for drug delivery through buccal route: Formulation and biointerface evaluation. Journal of Drug Delivery Science and Technology 68 (2022): 103030, 1-29.
24. Zaya, K. S., M. J. Nayak, and J. N. Mishra. Formulation and evaluation of floating tablet of atenolol for the treatment of hypertension. Journal of Population Therapeutics and Clinical Pharmacology 30, no. 4 (2023): 765-776.
25. Awandekar, Nishant B., Rita Tekade, Swati Dhawas, Supriya Gayakwad, and Milind J. Umekar. Formulation and evaluation of fast dissolving sublingual tablets of nifedipine using Plantago ovata husk as a natural superdisintegrant. Research Journal of Pharmacy and Technology 15, no. 2 (2022): 633-638.
26. Göbel, Anja, Jessica Bassi da Silva, Michael Cook, and Jörg Breitkreutz. Development of buccal film formulations and their mucoadhesive performance in biomimetic models. International Journal of Pharmaceutics 610 (2021): 121233.
27. Kottke, Dina, Haidara Majid, Jörg Breitkreutz, and Bjoern B. Burckhardt. Development and evaluation of mucoadhesive buccal dosage forms of lidocaine hydrochloride by ex-vivo permeation studies. International journal of pharmaceutics 581 (2020): 119293, 1-8.
28. Esim, Ozgur, Ayhan Savaser, Cansel Kose Ozkan, Cetin Tas, and Yalcın Ozkan. Investigation of the mucoadhesivity, swelling, and drug release mechanisms of indomethacin buccal tablets: effect of formulation variables. Drug Development and Industrial Pharmacy 46, no. 12 (2020): 1979-1987.
29. Saker, Ranim, Wehad Ibrahim, and Mohammad Haroun. Preparation and evaluation of nifedipine solid dispersions. Research Journal of Pharmacy and Technology 13, no. 9 (2020): 4148-4152.
30. Saeed, Adnan, Development and optimization of atenolol loaded polymeric microspheres: in vitro attributes. Lat Am J Pharm. 2020 Jan 1;39(4):707-16.
31. Pichayakorn, Wiwat, Pattwat Maneewattanapinyo, Kamon Panrat, Chaowalit Monton, and Jirapornchai Suksaeree. Formulation design of oral strip-films based on PVA/PVP polymer blends for nicotine delivery. Journal of Polymers and the Environment 30, no. 10 (2022): 4479-4491.
32. Ata, Sadia, Atta Rasool, Atif Islam, Ismat Bibi, Muhammad Rizwan, Muhammad Khalid Azeem, and Munawar Iqbal. Loading of Cefixime

to pH sensitive chitosan based hydrogel and investigation of controlled release kinetics. International journal of biological macromolecules 155 (2020): 1236-1244.

33. Vecchi, Camila Felix, Rafaela Said Dos Santos, and Marcos Luciano Bruschi. Technological development of mucoadhesive film containing poloxamer 407, polyvinyl alcohol and polyvinylpyrrolidone for buccal metronidazole delivery. Therapeutic delivery 11, no. 7 (2020): 431-446.
34. Viswanadh, Matte Kasi, Bioadhesive chitosan nanoparticles: dual targeting and pharmacokinetic aspects for advanced lung cancer treatment. Carbohydrate Polymers 274 (2021): 118617, 1-14.
35. Anothra, Pooja, Deepak Pradhan, Pradeep Kumar Naik, Goutam Ghosh, and Goutam Rath. Development and characterization of 5-fluorouracil nanofibrous film for the treatment of stomach cancer. Journal of Drug Delivery Science and Technology 61 (2021): 102219, 1-26.
36. Adimulapu, Anil K., Lalchand D. Devhare, Anasuya Patil, Nilesh O. Chachda, and G. Dharma Moorthy. Design and Development of Novel Mini Tablet Cap Technology for the Treatment of Cardiovascular Diseases. International Journal of Drug Delivery. Technology 13, no. 3 (2023): 801-806.