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Formulation, Optimization and *In-Vivo* Pharmacokinetic Evaluation of Carvedilol Mucoadhesive Buccal Films by Using Natural Polymers

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ABSTRACT

This study utilizes Quality by Design (QbD) principles to optimize carvedilol buccal film composition. The optimization method used a factorial design, changing three important material attributes (CMAs): the concentration of lime basil seed mucilage, the concentration of plasticizer (PG 10%), and the percentage of permeation enhancer at three different levels (low, medium, and high). Critical quality attributes (CQAs) included drug content (%), drug release percentage, and folding endurance. A 3^3 factorial experimental design created a first-order response surface model. The optimized buccal formulation, R20, featured high polymer, plasticizer, and medium permeation enhancer concentrations. It yielded favorable CQAs: 96.9 ± 2.4% drug content, 97.4 ± 2.4% drug release at 24 hours, and 422 ± 12 folding endurance. The identified optimal concentrations, established through the solvent casting process, offer a promising buccal film formulation for further research and potential *in vivo* studies.

***Keywords*:** Optimization, Factorial design, Quality by design, Critical quality attributes, Critical material attributes, Buccal film

Introduction

Despite the fact that the oral route has traditionally been the recommended method of medicine administration, many patients find it difficult to swallow tablets and capsules. To circumvent swallowing issues, a variety of therapeutic formulations have been developed, including oral gels, buccal pills, patches, and other forms of fast-dissolving drug delivery systems. Mucoadhesive buccal films are superior to standard oral dosage forms because they adhere to the buccal mucosa and distribute medications in a regulated manner, whether for transmucosal or local administration. The buccal transmucosal route is a non-invasive systemic administration channel that has various advantages over oral administration. Because of the extensive vascularization of the mucosa, it has a faster onset of action, avoids enzymatic degradation in the gastrointestinal tract, prevents first-pass metabolism, and improves bioavailability.[1]

Another advantage is that it is easily accessible to the oral cavity and buccal mucosa, which makes it more comfortable for patients and may lead to greater drug adherence.[2] However, saliva constantly scrubs the oral mucosa, and tongue and jaw movements may further impair the efficiency of a buccal medication delivery device.[3] Furthermore, drug permeability in the buccal mucosa is known to be lower than in the small intestine, but this may be compensated for by a longer residence duration. A mucoadhesive substance can be used to enhance the retention period on the buccal mucosa.

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Buccal films can be orodispersible, which means they dissolve quickly and are intended to be consumed, or mucoadhesive, which means they cling to the oral mucosa.[4] The qualities of the film influence whether the medicine released by it is absorbed transmucosally or in the gastrointestinal system. A single-layer or multi-layer mucoadhesive buccal film can provide extended release.[5] As oral patches, multi-layer films with a non-dissolvable layer that permits unidirectional drug release are often utilized, either for transmucosal absorption or for a local effect in the mouth cavity where absorption is required.[6] Hand removal of multi-layer films is extremely usual. Single-layer buccal films that erode over time can be utilized in conjunction with a traditional orodispersible fast-dissolving drug delivery vehicle.[7] The matrix polymers' hydration, swelling, and dissolving properties may determine how long an oromucosal buccal film remains on the oral mucosa. Buccal films must be able to dissolve a poorly soluble medication, as well as have wetting and disintegration capabilities, mucoadhesion impact, and improve a drug delivery platform.[8]

Carvedilol is an antiproliferative and antioxidant lipophilic adrenergic receptor antagonist that is nonselective. It has vasodilating properties, primarily related to the blocking of beta-1 receptors.[9] Carvedilol is commonly used to treat angina pectoris and mild to moderate hypertension and is often used in combination with other drugs.[10]

When selecting components and processing processes for a dosage form, physical, chemical, and biological properties must all be considered. The final product must meet bioavailability standards and the process and product repeatability criteria for mass production. It is crucial to understand the theoretical composition, target processing parameters, and the acceptable ranges for each component and processing parameter during formulation.[11]

The optimization method gives a complete grasp of formulation and processing features, allowing for parameter range exploration and justification. When selecting a formulation for a given product, qualitative techniques can be used first. Optimizing a formulation that may first be examined qualitatively is a useful approach of evaluation. The goal of optimization is perfection, efficacy, and functionality, which necessitates methodical techniques, careful variable management, and gradual tweaks until an ideal system is attained.[12]

Regardless of the initial construction procedure, the trial and error method improves the quality of the dosage form. Factorial design appears as a highly successful strategy for developing an appropriate mathematical model with the fewest necessary experiments in the context of formulation design optimization. In a factorial study, all components can be changed at the same time, allowing for the investigation of each variable's effects at different levels and their interrelationships.[13]

The primary aim of this study is to enhance the formulation of carvedilol buccal films through the application of Quality by Design (QbD) principles. This optimization process employs a factorial design approach, where Critical material attributes (CMA) including Polymer Solvent Concentration (specifically, Basil seed mucilage in percentage), Plasticizer Concentration (set at 10% Propylene Glycol), and Permeation Enhancer (expressed as a percentage of dimethyl sulfoxide or DMSO) are manipulated. By varying these CMAs, we intend to evaluate their impact on Critical quality attributes (CQA) such as the percentage of Drug Content, the percentage of Drug Release, and Folding Endurance. This systematic approach enables us to discern the optimal conditions for achieving the desired attributes, ultimately leading to an improved formulation of carvedilol buccal films.

Materials and Methods

Carvedilol was provided by Swarnoop Pharmaceuticals (Pune, India). Polymers such as hydroxy propyl methyl cellulose, crosspovidone, poly vinyl pyrolidone, propylene glycol, dimethyl sulfoxide and ethanol were supplied by Loba Chemie (Mumbai, India). The rest of the components were of pharmaceutical and analytical quality.

Fabrication of Mucoadhesive Buccal Films

The most commonly employed technique for producing Carvedilol mucoadhesive buccal films is the solvent casting method. In this approach, pre-lubricated petriplates serve as substrates, and various concentrations of natural polymers such as *Lime Basil seeds*, *Sweet basil*, and *Purple basil mucilage* were utilized, as detailed in Table 1. Additionally, the impact of the selected natural polymer, Basil seed mucilage, was compared to that of the synthetic polymer Carbopol 934 P, as presented in Table 2. Through screening design, it was determined that a 1.5% w/v Basil seed mucilage solution was the most suitable polymer, subsequently utilized in the optimization process. The process involved dispersing the estimated amount of polymer in 50% v/v ethanol. Following this, 30% w/w propylene glycol (PG) was added as a plasticizer, along with the required quantity of the permeability enhancer, Dimethyl Sulfoxide (DMSO). A precise 10 mg of Carvedilol was weighed (Schimadzu, Japan) and added to the polymeric solutions. To attain a homogeneous, viscous consistency, the solution was mixed using a magnetic stirrer at 60 RPM. Subsequently, the solution underwent sonication in a bath sonicator (REMI, Mumbai) for approximately 5 minutes to eliminate any air bubbles. The resulting polymer solution was then cast onto a greased 4.5 cm diameter petriplate, covered with a funnel to prevent solvent evaporation, and allowed to air dry overnight at room temperature. The dried films were carefully removed and shielded with aluminum foil, then preserved in desiccators until further research was conducted.[14-16]

Table 1: Screening studies effect of various natural polymers on buccal tablets

Formulation code	Drug	Natural Polymers mucilage (%)			Solvents (mL)			Evaluation parameter	
	Carvedilol	*Lime Basil seeds*	*Sweet basil*	*Purple basil*	Ethanol (50% v/v)	PG (30%w/w)	DMSO (5%W/V)	% DC	MS
P1	10	0.5	-	-	10.0	0.5	0.25	68.60 ± 2.4	20.5 ± 1.8
P2	10	1.0	-	-	10.0	0.5	0.25	76.42 ± 2.2	26.4 ± 1.2
P3	10	1.5	-	-	10.0	0.5	0.25	84.50 ± 2.8	30.6 ± 1.8
P4	10	-	0.5	-	10.0	0.5	0.25	46.66 ± 2.4	11.8 ± 1.4
P5	10	-	1.0	-	10.0	0.5	0.25	53.40 ± 3.2	15.4 ± 2.6
P6	10	-	1.5	-	10.0	0.5	0.25	54.42 ± 3.0	18.6 ± 1.8
P7	10	-	-	0.5	10.0	0.5	0.25	63.40 ± 3.4	12.4 ± 1.6
P8	10	-	-	1.0	10.0	0.5	0.25	68.66 ± 3.2	13.8 ± 1.8
P9	10	-	-	1.5	10.0	0.5	0.25	72.48 ± 3.8	14.4 ± 2.2

DC: Percent Drug Content, MS: Mucoadhesive Strength; PG: Propylene glycol; DMSO: Dimethyl Sulfoxide

Table 2: Screening of Natural Vs. Synthetic polymers in Carvedilol Buccal films (mean ± SD, n=3)

Formulation code	Drug (mg)	Polymers (%)			Solvents (mL)			Evaluation parameter	
	Carvedilol	*Lime Basil Seed mucilage*	PVP	Carbopol 934	Ethanol (50% v/v)	PG (30%w/w)	DMSO	% DC	MS
F1	10	0.5	-	0.5	10.0	0.5	0.25	54.42 ± 2.4	18.8 ±1.2
F2	10	1.0	-	0.5	10.0	0.5	0.25	68.60 ±2.2	20.5 ±1.6
F3	10	1.5	-	0.5	10.0	0.5	0.25	92.48 ±2.4	32.5 ±2.2
F4	10	2.0	-	0.5	10.0	0.5	0.25	93.40 ±2.8	32.8 ±1.4
F5	10	2.5	-	0.5	10.0	0.5	0.25	94.50 ±3.2	33.4 ±2.6
F6	10	-	0.5	0.5	10.0	0.5	0.25	46.66 ±3.2	10.6 ±1.4
F7	10	-	1.0	0.5	10.0	0.5	0.25	53.40±2.2	14.3 ±2.6
F8	10	-	1.5	0.5	10.0	0.5	0.25	72.66±2.4	16.4 ±1.6
F9	10	-	2.0	0.5	10.0	0.5	0.25	76.42±2.6	22.8 ±1.4
F10	10	-	2.5	0.5	10.0	0.5	0.25	82.72±2.4	23.4 ±2.6

DC: Percent Drug Content, MS: Mucoadhesive Strength; PG: Propylene glycol; DMSO: Dimethyl Sulfoxide; PVP: Polyvinyl Pyrolidone

Design of 3^3 factorial designs

A 3^3 factorial experimental design consisting of 27 runs was implemented to apply a first-order response surface model and conduct result analysis. To determine suitable polymer solution concentrations, initial screening tests were conducted. Based on a preparatory study, preliminary concentration ranges for three variables were established as follows: 0.5%, 1%, and 1.5% for polymer solution concentration, 0.25%, 0.5%, and 0.75% for plasticizer concentration, and 0.5%, 1.0%, and 1.5% for permeation enhancer concentration. Subsequently, these concentration ranges were further refined and optimized using a 3^3 factorial experimental design with the assistance of JMP Design Expert software. This particular configuration allowed for the assessment of the relationships between the studied parameters and their impacts on the measured responses. The parameters investigated included X1 (Polymer concentration, Basil seed Mucilage), X2 (Plasticizer Concentration, PG 10% in % w/v), and X3 (Permeation enhancer, DMSO, in % w/v), each at three different levels coded as low (1), medium (2), and high (3). The responses of interest encompassed Y1 (Drug Content in %), Y2 (Percentage of drug release), and Y3 (Folding endurance). In summary, the 3^3 factorial experimental designs was employed to systematically explore the influence of these parameters on the measured responses.[17-20]

Analyzing Factorial experiment

Physicochemical evaluation of buccal films

The following are the physicochemical characteristics of the generated buccal films:

Thickness

The thickness of each film was determined at six distinct locations on the film with a digital vernier caliper (Mitutoyo, India) and the average thickness was calculated. [21]

Weight of films

After weighing 10 separate films with the identical formulation on a digital scale, the mean of three films was determined. [22]

Folding endurance

Individual films from all compositions were folded frequently till they ruptured at same location in folding endurance testing. The number of times the film could be folded in the same spot without breaking was used to calculate the folding endurance, and the average of three films was used. [23] It was calculated using the equation 1.

$$Folding\ Endurance = \frac{Total\ No.of\ folds}{Number\ of\ films\ tested} \quad \text{(Eqn. 1)}$$

Percentage moisture absorption (PMA)

The assessment of the physical stability of buccal films in a high-humidity environment was conducted through a percent moisture absorption test. In this test, three buccal film samples, each measuring 1 cm, were carefully cut and precisely weighed to establish their initial weights. These films were then placed inside desiccators containing a saturated solution of aluminum chloride, creating and maintaining a controlled high-humidity environment set at 75% relative humidity (RH). After an exposure period of three days, the films were removed from the desiccators and weighed once again to determine their final weights. To quantify the extent of moisture absorption, the percent moisture absorption for each buccal film sample was calculated using the equation 2:

$$Percentage\ Moisture\ Absorption = \frac{Final\ weight - Initial\ weight}{Initial\ weight} X100 \quad \text{(Eqn. 2)}$$

To derive a comprehensive understanding of moisture absorption behavior, the average percent moisture absorption was computed by summing the individual percent moisture absorption values for the three films and dividing by 3, which represents the number of films tested. This test provides valuable insights into the buccal films' physical stability under high-humidity conditions, offering critical data on their propensity to absorb moisture over the specified exposure period. [24,25]

Percentage moisture loss (PML)

The percent moisture loss test is employed to assess the integrity of the film under dry conditions. To conduct this test, three 1 cm film samples were meticulously cut and then placed inside desiccators containing fused anhydrous calcium chloride. Their initial weights were precisely recorded. Following a three-day exposure period, the films were removed from the desiccators and weighed again to establish their final weights. To quantify the extent of moisture loss, the percent moisture loss for each film sample was calculated using the equation 3:

$$Percentage\ Moisture\ Loss = \frac{Initial\ weight - Final\ weight}{Initial\ weight} X100 \quad \text{(Eqn. 3)}$$

To obtain a comprehensive assessment of moisture loss behavior, the average percent moisture loss was determined by summing the individual percent moisture loss values for the three films and then dividing by 3, representing the number of films tested. This test offers valuable insights into the film's ability to maintain its structural integrity under dry conditions and provides crucial data regarding its moisture loss characteristics.[26]

Swelling percentage (%S)

A Carvedilol buccal film was transferred to a clean petridish with 50 mL of phosphate buffer at pH 6.8. The weight of the film was estimated by adding 15 minutes to it at a time for 60 minutes. The proportion of swelling was calculated using equation 4 as follows.[27]

$$\%\ S = \frac{X_t - X_0}{X_0} X100 \quad \text{(Eqn. 4)}$$

Where, % S - swelling percentage; Xt - the weight of swollen film after time t; X0 -weight of film at zero time.

Drug content estimation

Each formulation's buccal film was cut into three equal pieces and put in a 100 mL phosphate buffer (pH6.8). The materials were agitated for up to 24 hours before being filtered. The absorbance was measured at 240 nm with a UV Spectrophotometer (Schimadzu, Japan) after the filtrate was diluted as needed. Three films were averaged to determine the drug content.[28]

Measurement of buccoadhesive strength

A modified balance approach was used to assess *ex vivo* buccoadhesive strength. Within 2 hours following killing, fresh sheep buccal mucosa was acquired and used. By removing the underlying fat and weak tissues, the mucosal membrane was eliminated. The membrane was moistened with isotonic phosphate buffer (IPB) pH 6.8 after being washed with distilled water. Sheep buccal mucosa was applied to the plane surface of a glass slide affixed to the base of a smaller beaker (using adhesive tape) then inverted in a 500 mL beaker attached to the bigger beaker. Isotonic phosphate buffer pH 6.8 was added to the inverted beaker with buccal mucosa lifted to the surface. With cyanoacrylate adhesive, the buccal film was attached to the bottom surface of the top clamp. For initial hydration and swelling, the exposed patch surface was moistened with phosphate buffer and left for 30 seconds. The platform was then carefully elevated till the surface of the film made contact with the mucosa. A weight was added on the right hand pan prior to the test to ensure that both sides of the balance were equal. The patch across the mucosa was reduced after a 5 g weight was removed from the right hand pan. For a total of 5 minutes, the balance was held in this position.[29] The force of adhesion was calculated using the equation 5.

$$Force\ of\ adhesion\ (N) = [Bioadhesive\ strength\ (g) \times 9.8]/1000 \quad \textit{(Eqn 5).}$$

Measurement of mechanical strength

The mechanical strength of Carvedilol buccal films was measured using custom-built equipment that comprised a microprocessor force gauze connected to a motor, as well as a stand and cell. Cutouts of 20 mm diameter films with minimum obvious damage were placed at a spacing of 3 cm between two clams. During the experiment, the two

clamps are positioned in such a way that they should not injure the film. The bottom clamp was locked in place, and the top clamp was pushed at a 2 mm/sec speed until the film shattered. Following that, the broken point was recorded, followed by extension. The tensile strength and elongation at break were calculated using equation 6 and 7. [30,31]

$$Tensile\ strength\ (kg.mm^2) = \frac{Force\ at\ break\ (kg)}{Inital\ cross\ sectional\ area\ of\ the\ sample\ (mm^2)} \quad \text{(Eqn. 6)}$$

$$Elongation\ at\ break\ (\%mm^2) = \frac{Increase\ in\ length\ (mm)}{Original\ length\ (mm)} X \frac{100}{Cross\ sectional\ area\ (mm^2)} \quad \text{(Eqn. 7)}$$

In vitro drug release studies

The *in vitro* release studies were performed in phosphate buffer solution (pH 6.8, 100 mL) at 37ºC using a modified dissolving apparatus. The receptor compartment is a 250 mL beaker, while the donor compartment is an open end tube. A magnetic stirrer assembly with a temperature-controlled hot plate was used in the experiment. The temperature of the dissolving media in the 100 mL container was kept at 37°C. A semi-permeable barrier separated the donor compartment from the medium, which was filled with film. After that, the donor tube was placed in the dissolving medium (receptor compartment), which was held at 37°C and agitated at 100 rpm with a magnetic stirrer. One millilitre samples were obtained at predetermined times. For each sample removed, an equivalent amount of phosphate buffer was added to the dissolving media to maintain a consistent volume and sink condition. The diluted solutions were then spectrophotometrically evaluated at 240 nm, and each of the removed samples was tenfold diluted.[32]

In vitro drug release kinetics studies

Data from *in vitro* release was fitted into multiple equations and kinetic models to explain the release kinetics of Carvedilol from buccal films. The kinetic models used were zero-order, Higuchi, and Peppa's. The results of the buccal film formulations were plotted in a variety of kinetic models, including cumulative percentage drug release Vs square root of time (Higuchi's) and log cumulative percentage release Vs log time (Peppas). To identify the process of Carvedilol release from buccal films, the release data was fitted into Higuchi's models. Namely Zero order: $Q=K_0t$; Higuchi's square rate at time: $Q=KHt_{1/2}$ and Peppas: $F=Kmtn$, where Q is amount of drug release at time t, F is Fraction of drug release at time t, K_0 is zero order kinetic drug release constant, KH is Higuchi's square root of time kinetic drug release constant, Km is constant incorporating geometric and structural characteristic of the films and n is the diffusion exponent indicative of the release mechanism. The correlation coefficient values (r) from Higuchi's model indicate the kinetic of drug release and diffusion exponent values (n) from Peppas model indicate the mechanism of drug release. [32]

To clarify and provide equations for each of these models:

Zero-Order Model ($\boldsymbol{Q = K0t}$) (Eqn. 8)

In this model, the rate of drug release is constant over time. Q represents the amount of drug released at time t. K0 is the zero-order kinetic drug release constant, which indicates the rate of drug release. t is the time.

Higuchi's Model ($\boldsymbol{Q = KHt0.5}$) (Eqn. 9)

Higuchi's model describes drug release as a square root of time-dependent process. Q represents the amount of drug released at time t. KH is Higuchi's square root of time kinetic drug release constant. t is the time raised to the power of 0.5, indicating the square root of time.

Peppas' Model ($\boldsymbol{F = Kmtn}$) (Eqn. 10)

Peppas' model is used to describe drug release from polymeric systems and can provide insights into the release mechanism. F represents the fraction of drug released at time t. Km is a constant that incorporates geometric and structural characteristics of the films. n is the diffusion exponent, which indicates the mechanism of drug release. Different values of "n" correspond to different release mechanisms, such as Fickian diffusion ($n < 0.5$), non-Fickian or anomalous diffusion ($0.5 < n < 1$), or Case II transport ($n = 1$). The correlation coefficient values (r) obtained from Higuchi's model can help assess the kinetic nature of drug release. A high "r" value indicates a good fit to the Higuchi model, suggesting a square root of time-dependent release. The diffusion exponent (n) obtained from Peppas' model provides information about the mechanism of drug release. Depending on the value of "n," you can infer whether the release follows Fickian diffusion ($n < 0.5$), non-Fickian diffusion (anomalous release, $0.5 < n < 1$), or Case II transport ($n = 1$). These models and their associated parameters are valuable tools for understanding the release kinetics and mechanisms of drug release from buccal films and can aid in the formulation and optimization of drug delivery systems.

Ex vivo permeation study through sheep buccal mucosa

In an *ex vivo* permeation study with Carvedilol in a modified diffusion cell at 37°C, a fresh sheep buccal mucosa was employed. Fresh sheep buccal mucosa was inserted between the donor and receptor compartments. Sheep buccal mucosa was affixed to one end of a donor compartment, which is an open-ended cylinder. The film should be applied to the mucosal membrane in such a way that it adheres to it. The pH 6.8 IPB was poured into the receptor chamber. The mixture was kept at 37 degrees Celsius and magnetically stirred. Samples were taken at regular intervals and evaluated with a UV Spectrophotometer set to 240 nm. [33]

In vivo pharmacokinetic Studies

Rats A total of 24 male Wistar rats (240–260g) were obtained from the animal house in the Sastra college of Pharmaceutical Education and Research, AP, India. The research protocol and ethical guidelines of the Sastra college of Pharmaceutical Education and Research, AP, India Research Ethics Committee were strictly followed (approval reference IAEC/SCPER/2021-22/12). All rats had unrestricted access to water and a normal rodent diet. Rats were randomly divided into three groups, each contains six rats to test the absorption of a single dose of 0.5 mg of dosage form administered by three different pharmaceutical forms: (a) Oral group (OG) where an oral dose of 0.5mg of Carvedilol was ingested via gastric gavage after intraperitoneal (IP) anaesthesia. (b) Carvil (0.5mg) PO was inserted at the buccal cavity of the anesthetized rat. (d) Carvedilol buccal film equivalent to 0.5mg dose was inserted at the buccal cavity of the rat.[34-36]

Dosage and Blood Sampling

The dorsal hub of the catheter was fixed to the harness which was fitted around the neck and forelimbs of the rat. Designed Buccal films (R20) with 0.5 mg dosage were inserted in the buccal cavity, respectively. Serial blood samples (200 µL/each) were withdrawn at 7 time-points (1, 2, 4, 8, 12, 18 and 24 h) from tail vein. Blood samples were collected in heparinized mini collection tubes, centrifuged then the plasma was kept frozen until the time of analysis.[34-36]

Quantification of Carvedilol in Plasma

Quantification of Carvedilol in Plasma was carried out by using HPLC (Schimadzu, Japan). The column used is reversed phase C18 column (250 mm X 4.6 mm i.d., Particle size - 5 µm) with the mobile phase of mixture of acetonitrile and 0.1% formic acid (70:30, v/v) with an flow rate of 1 mL/min and an injection volume of about 5 µl. Wavelength maxima of Carvedilol was Detected by performing the following procedure and its criteria was the run time of 10 - 20 min and sample was detected at 241nm. Calibration curve was calibrated for 8 solutions of Carvedilol in phosphate buffer pH 7.4 with the concentration ranging from 0.02-0.16 µg/mL by UV-Vis detection at 241 nm.[34-36]

Pharmacokinetic data analysis

The time-versus-plasma drug concentration data obtained through HPLC analysis were plotted using PK solver software (PKTool v2.0). From these individual plasma concentration-time profiles, essential pharmacokinetic parameters such as peak plasma concentration (Cmax), the time taken to reach Cmax (tmax), area under the

concentration-time curve from 0 to the last measurable time point (AUC_{0-t}), and area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) were directly determined. Furthermore, additional pharmacokinetic parameters including biological half-life ($t_{1/2}$) and Mean Residence Time (MRT) were computed using PK solver software. Statistical analysis involved the evaluation of differences in various pharmacokinetic parameters. This was achieved through ANOVA, where the interval of the ratio of test/reference was calculated using log-transformed data. In the realm of pharmacokinetic analysis, the evaluation of essential response variables like Cmax, tmax, AUC0–∞, and MRT serves as a crucial indicator of bioavailability enhancement achieved by the formulated dosage form. This assessment involved comparing the pharmacokinetics of each treatment group with the control group, employing an analysis of variance (ANOVA) within a crossover design framework. Additionally, a pivotal aspect of this analysis was the calculation of 90% confidence intervals for the ratio of test/reference, based on log-transformed data.[34-36]

Statistical analysis

To ascertain the statistical significance of the formulated dosage form, specific criteria were applied: Firstly, the formulation was considered statistically significant when the difference (p-value) between the treatment parameters of two compared groups was less than 0.05, and the confidence interval for these parameters fell within 95%. This signified a substantial improvement attributed to the formulation. Conversely, if the difference (p-value) between two compared treatment parameters was greater than 0.05, and the confidence interval for these parameters fell within 85%, the formulation was deemed statistically insignificant, indicating that it did not bring about a significant alteration in bioavailability. These comprehensive statistical assessments were carried out using Graph Pad Prism version 5 for Windows, a software tool provided by Graph Pad Software, San Diego, Calif., USA.[34-36]

Results and Discussion

Selection of polymer by screening studies

The preliminary screening studies successfully identified a suitable polymer for the development of buccal films in the trial formulations. The solvent casting technique was employed to create nine buccal films, each differing in terms of the types and concentrations of natural polymers. These polymers were extracted from Basil seeds, Sweet basil, and Purple basil through a hydroalcoholic (50:50 % V/V) maceration extraction process.

Among the formulations, P3, comprising 1.5 mg of basil seed mucilage as the film-forming polymer, emerged as the optimal choice. This selection was based on its impressive % Drug Content (%DC) of approximately 84.50 ± 2.8% and a robust Mucoadhesive Strength (MS) measuring around 30.5 ± 1.8. Consequently, in the pursuit of cost-effectiveness, the decision was made to proceed with 1.5 mg of Basil seed mucilage as the preferred natural polymer due to its economic advantage. This polymer was chosen for further optimization. It's worth noting that formulations involving Tamarind and Purple basil exhibited lower %DC and MS in comparison. So it was not selected as the good polymer for the further formulation of buccal film.

Discussion on Optimization:

Table 3 and Figure 1 show the effect of CMA attributes such as [Polymer solvent Concentration (*Basil seed* mucilage) in various percent w/v; Plasticizer Concentration (PG 10%) in percent w/v; Permeation enhancer [DMSO] (percent w/v)] on CQA variables such as [percent Drug content, percent Amount of Drug release, Folding endurance].

Response of CMA on % Drug Content (DC)

The mean DC for all formulations are shown in Table 4. The DC for all Carvedilol Buccal Film formulations was typically found to be in the range of 54.2 ± 2.4 to 98.4 ± 2.2 %. The variation in DC is mainly based on the influence of a CMA (Polymer concentration). The formulation run R1, R14, R15, R16, R18, R20, R21 shows % DC greater than 85% as per required constraint. However, according to the acceptance constraint, the formulation runs R16 (3 mL of X1 factor; 0.75 % of factor X2 and 1.5 % of factor X3) and R20 (3 mL of X1 factor; 0.75 % of factor X2 and 1 % of factor X3) show %DC values of about 96.4 ± 2.2 and 96.9 ± 2.2 %, respectively, based on the other CQA parameters. The remaining formulation yields a lower percentage of DC than expected. As a result, polymer concentration had a significant impact on percent DC. The results were analyzed using the F-test, p value at 95 % confidence, and coefficient of determination, and the statistical analysis of the data revealed that the model's fitness to the data was meaningful at p value <0.05. P value 0.0000 for the influence of polymer concentration on % DC. As a result, the desired model's shows a strong regression (R^2) value. The F-test in ANOVA was used to calculate the P-value for response percent DC with P values 0.0001. There was a greater degree of similarity between expected and observed values. The impact of CMA (Polymer concentration) on CQA (percent DC) may be determined by analyzing Response surface profiler and Contour profilers plots. Polymer concentration (X1) demonstrates an increase in percent DC among the CMAs. The percent DC polynomial regression equation is as follows:

$$Y1 = 38.81 + 14.83\ X1 \quad \text{(Eqn. 8)}$$

Table 3: Absolute values of levels of CMA employed in 3^3factorial design

CMA		**Levels**			
		Coded	**1**	**2**	**3**
Polymer solvent Concentration (*Lime Basil seed mucilage*) in %w/v		X1	0.5	1	1.5
Plasticizer Concentration (PG 10%) in % w./v		X2	0.25	0.5	0.75
Permeation enhancer [DMSO] (% w/v)		X3	0.5	1.0	1.5
Carvedilol – 10mg in all formulation					
Ethanol (50% v/v) – 10 mL in all formulation					
Response		Constraint			
Y1	% Drug Content	Maximum >85%			
Y2	% Amount of drug release at 24 hrs	Maximum >85 %			
Y3	Folding Endurance	Maximum >350			

CMA: Critical Material Attributes

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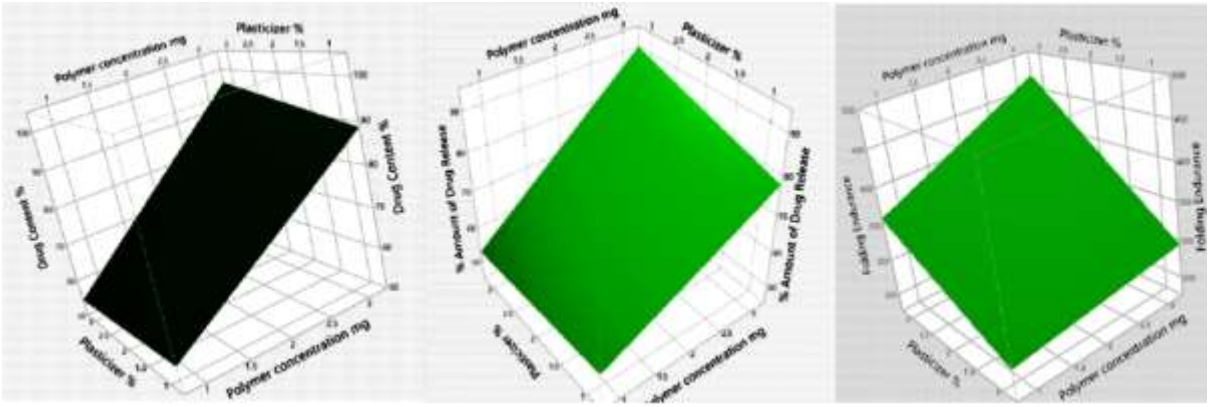

Figure 1: Response Surface Profiler graph showing the relation between CMA Vs. CQA; (a) CMA Vs. % Drug content plot; (b) CMA Vs. % Amount of Drug release; (c) CMA Vs. Folding endurance

Table 4: Optimization of Carvedilol Buccal Film formulation by 3^3 factorial design and effect of CMA on CQA (*mean ± SD, n=3)*

Run	CMA			CQA		
	X1	X2	X3	% Drug Content	% Amount of drug release at 24 hour	Folding Endurance
R1	3	2	1	98.4 ± 2.2	74.2 ± 2.0	386 ± 14
R2	2	2	1	76.8 ± 2.8	66.8 ± 2.4	354 ± 12
R3	1	1	2	60.2 ± 2.6	58.4 ± 2.6	268 ± 10
R4	1	3	1	54.2 ± 2.4	52.4 ± 2.4	366 ± 12
R5	2	2	2	68.4 ± 2.8	66.8 ± 2.4	302 ± 14
R6	2	3	1	64.2 ± 2.2	62.6 ± 2.8	368 ± 16
R7	2	1	3	72.8 ± 2.8	70.6 ± 2.2	296 ± 12
R8	1	3	3	74.6 ± 2.2	70.8 ± 2.4	384 ± 10
R9	1	2	2	62.8 ± 2.2	60.6 ± 2.6	286 ± 14
R10	2	3	3	70.2 ± 2.6	68.4 ± 2.4	398 ± 12
R11	1	1	3	58.8 ± 2.2	56.8 ± 2.2	284 ± 14
R12	1	2	3	59.4 ± 2.8	57.8 ± 2.4	304 ± 16
R13	2	1	1	72.8 ± 2.6	69.6 ± 2.2	292 ± 12
R14	3	2	2	97.6 ± 2.2	90.8 ± 2.4	442 ± 16
R15	3	1	2	86.4 ± 2.6	82.4 ± 2.4	290 ± 12
R16	3	3	3	92.4 ± 2.2	92.8 ± 2.2	464 ± 18
R17	2	3	2	71.6 ± 2.4	69.8 ± 2.6	430 ± 16
R18	3	1	3	92.2 ± 2.2	74.6 ± 2.2	296 ± 12
R19	3	1	1	74.6 ± 2.6	68.6 ± 2.8	302 ± 16
R20	3	3	2	97.8 ± 2.2	97.4 ± 2.4	422 ± 12
R21	3	3	1	86.4 ± 2.8	74.6 ± 2.2	402 ± 14
R22	2	2	3	60.2 ± 2.4	54.6 ± 2.4	336 ± 16
R23	1	2	1	68.6 ± 2.8	65.8 ± 2.6	366 ± 14
R24	2	2	3	56.4 ± 2.2	53.6 ± 2.4	346 ± 12
R25	1	3	2	58.8 ± 2.0	54.2 ± 2.2	302 ± 16
R26	3	2	3	83.4 ± 2.8	78.4 ± 2.8	367 ± 18
R27	2	1	2	74.6 ± 2.6	68.8 ± 2.6	294 ± 16

CMA: Critical material attributes CQA: Critical quality attributes

Response of CMA on % amount of drug release (%ADR)

Table 4 shows the average % ADR for all formulations. For all Carvedilol Buccal Film formulations, the % ADR was commonly found to be in the range of 52.4 ±2.4 to 94.6±2.2 percent. The difference in % ADR is primarily due to the control of a CMA (Polymer concentration, Permeation enhance concentration). As needed by the constraint, the formulation runs R14, R16 and R20 show a % ADR more than 85 %. However, based on the other CQA parameters, the formulation runs R16 (3 mL of X1 factor; 0.75 % of factor X2 and 1.5 % of factor X3) and R20 (3 mL of X1 factor; 0.75 % of factor X2 and 1 % of factor X3) show % ADR values of around 97.8 ± 2.2 and 92.4 ± 2.4 %, respectively, according to the acceptance

constraint. In the remaining formulation, the % ADR is lower than the desired constraint values. As a result, polymer concentration and permeation enhancers had a significant impact on % AMT. The results were interpreted using the F-test, p value at 95 % confidence Interval, coefficient of determination and the statistical analysis of the data revealed that the model's fitness to the data was meaningful at p value < 0.05. The effect of polymer concentration on % DC shows P value 0.0000. As a result, the desired model's strong regression (R2) value was created. The F-test in ANOVA was used to assess the P-value for response percent %ADR, with P values <0.0001. There was a greater degree of similarity between expected and observed values. The effect of CMA (polymer concentration and permeation enhancer concentration) on CQA (% ADR) may be assessed using the Response surface profiler and Contour profilers plots. Polymer concentration (X1) and Permeation enhancer (X3) are two CMAs that exhibit an increase in % ADR. The % ADR polynomial regression equation is as follows:

$$Y2 = 34.62 + 11.85\ X1 + 3.34\ X3 \quad \text{(Eqn. 9)}$$

Response of CMA on folding endurance (FD)

Table 4 shows the average FD for all formulations. The formulations of FD all Carvedilol Buccal Film were commonly determined to be in the range of 268 ±10 to 442 ± 16. The control of a CMA is mostly responsible for the deviation in % ADR (Polymer concentration, Permeation enhance concentration). As per the specified constraint, all of the formulation runs indicate good FD values >350 %. However, based on the other CQA parameters, the formulation runs R16 (3 mL of X1 factor; 0.75 % of factor X2 and 1.5 % of factor X3) and R20 (3 mL of X1 factor; 0.75 % of factor X2 and 1 % of factor X3) have very good FD values of around 442 ± 16 and 420 ±12 %, respectively, according to the acceptance constraint. Remaining formulation reveals less FD than desired constraint values. Thus the concentration of polymer and concentration of plasticizer showed a substantial impact on FD values. The statistical analysis of the data showed that the fitness of the model to the data was meaningful at p value < 0.05 and the results were interpreted on the basis of the F-test, p value at 95 percent CI, coefficient of determination. The effect of polymer concentration on % DC shows P value <0.0000. Hence, strong regression (R^2) value of the desired model was designed. The P-value for response FD was calculated by the F-test in ANOVA which shows the P values <0.0001. Closer similarity between expected values and observed values was found. The impact of CMA (Polymer concentration and plasticizer concentration) on CQA (%ADR) can be measured through the analysis of Response surface profiler, Contour profilers plots. Among the CMAs, Polymer concentration (X1) and Plasticizer concentration (X2) shows an improvement of FD. The polynomial regression equation for FD is derived as equation

$$Y3 = 179.66 + 27.38\ X1 + 53X2 \quad \textit{(Eqn. 10)}$$

Physico-chemical evaluation of Carvedilol buccal films

The other Physico-chemical properties Mechanical strength (kg/mm^2); Thickness (mm); Weight (mg); Surface pH; % Swelling index (S); Percentage moisture absorption (PMA); Percentage moisture loss (PML) was evaluated and the data are shown in Table 5. The mechanical strength of all Carvedilol Buccal Film formulations were typically found to be in the range of 5.24±0.24 to 15.64±0.46. The thickness of all Carvedilol Buccal Film formulations were typically found to be in the range of 0.20 ±0.01 to 0.78 ±0.02.The weight of all the buccal film was in the range of 101.17±1.70 to 164.12±1.16. The pH of the buccal film was measured between 6.60±0.02 and 6.82±0.02, the % swelling of carvedilol buccal film was measured between 62.70±0.72 and 138.24±0.80 %, and the PMA of all formulations was determined between 3.56±0.25 and 11.64±0.12. The PML for all of the formulations ranged from 0.94±0.10 to 1.88±0.02. When 27 formulations were compared, R20 and R16 provided the best results in terms of the desired constraints, with mechanical strengths of 15.64±0.46 and 15.42±0.46, respectively. Thickness of R20 and R16 was found to be 0.78 ±0.02 and 0.78 ±0.01mm, Weight (mg) of R20 and R30 was found to be 154.53±0.80 to6.72±0.02, The PMA for R20 and R16 was shown in 124.2±0.99 and 3.56±0.25 and the PML was found to be 0.94±0.12 to 1.84±0.08. As a result of the data, it was determined that formulation R20 and R30 were the best formulations. When comparing the percentage amount of drug release between commercialized Coreg ER and Carvedilol Buccal Film (R20 and R16) (Figure 2), it was determined that the R20 formulation was the best since it showed a very good cumulative amount of drug release, 97.4±2.4 % at 24 hours.

In general, drug release from the R20 formulation followed a predefined release control pattern that followed a zero order drug release pattern. As shown in Figure 3, the *in vitro* release kinetics of Carvedilol buccal injection were assessed by fitting drug release data into various kinetic models such as First order, Zero order, Higuchi, Hixson Crowell, and Korsmeyer Peppas equations. The drug release kinetic data for an optimized formulation R20 followed the Zero order release kinetic model, with high linearity and a regression r^2 value of 0.954.

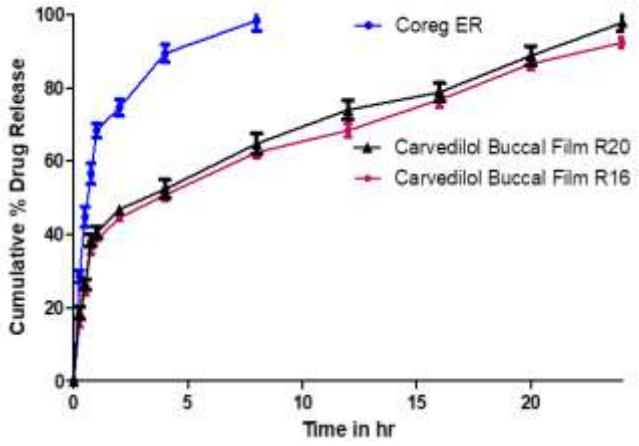

Figure 2: Comparative *in vitro* drug release of Carvedilol buccal film Vs. Coreg ER®

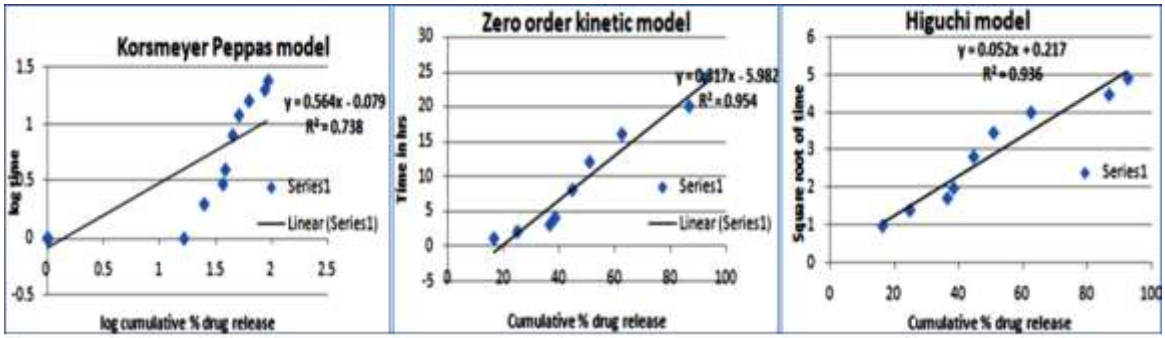

Figure 3: Release kinetics values of Carvedilol Buccal patches (R20)

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Table 5: Physicochemical evaluation of Carvedilol buccal films

Run	Mechanical strength (kg/mm^2)	Thickness (mm)	Weight (mg)	Surface pH	% S	PMA	PML
R1	11.74 ± 0.24	0.64 ± 0.02	140.42 ± 1.10	6.64 ± 0.04	99.62 ± 0.69	5.24 ± 0.06	0.94 ± 0.12
R2	5.54 ± 0.64	0.24 ± 0.01	102.45 ± 1.10	6.60 ± 0.02	67.50 ± 0.65	7.32 ± 0.10	1.14 ± 0.72
R3	5.24 ± 0.24	0.22 ± 0.02	104.37 ± 1.10	6.69 ± 0.02	69.70 ± 0.72	9.24 ± 0.12	1.54 ± 0.10
R4	5.46 ± 0.34	0.20 ± 0.01	102.94 ± 1.60	6.72 ± 0.02	71.62 ± 0.62	10.32 ± 0.14	1.14 ± 0.20
R5	8.66 ± 0.44	0.34 ± 0.02	122.23 ± 0.91	6.64 ± 0.04	78.62 ± 1.02	10.13 ± 0.24	1.08 ± 0.03
R6	8.62 ± 0.46	0.38 ± 0.01	120.37 ± 0.60	6.66 ± 0.04	82.64 ± 1.12	5.21 ± 0.12	1.18 ± 0.02
R7	10.64 ± 0.52	0.48 ± 0.02	132.93 ± 1.55	6.80 ± 0.02	97.42 ± 0.72	4.86 ± 0.26	0.94 ± 0.10
R8	10.47 ± 0.64	0.46 ± 0.01	132.18 ± 0.91	6.72 ± 0.04	92.53 ± 0.62	5.18 ± 0.28	0.98 ± 0.08
R9	9.66 ± 0.74	0.38 ± 0.02	128.53 ± 0.80	6.79 ± 0.06	82.4 ± 1.04	6.34 ± 0.34	1.12 ± 0.07
R10	9.54 ± 0.88	0.36 ± 0.02	130.31 ± 0.58	6.71 ± 0.02	80.4 ± 1.04	6.12 ± 0.22	1.06 ± 0.06
R11	6.45 ± 0.54	0.28 ± 0.02	112.37 ± 0.80	6.68 ± 0.02	62.70 ± 0.72	8.24 ± 0.24	1.21 ± 0.06
R12	6.56 ± 0.66	0.26 ± 0.01	112.17 ± 1.70	6.72 ± 0.04	63.70 ± 0.72	10.02 ± 0.23	1.10 ± 0.08
R13	9.42 ± 0.70	0.42 ± 0.02	130.07 ± 0.90	6.68 ± 0.02	89.60 ± 0.72	10.12 ± 0.22	1.12 ± 0.01
R14	15.23 ± 0.46	0.70 ± 0.01	166.12 ± 1.12	6.66 ± 0.04	104.9 ± 0.90	3.66 ± 0.10	0.98 ± 0.02
R15	14.62 ± 0.60	0.72 ± 0.02	140.22 ± 1.10	6.68 ± 0.06	132.4 ± 0.60	3.42 ± 0.22	0.96 ± 0.52
R16	15.42 ± 0.46	0.78 ± 0.01	164.12 ± 1.16	6.70 ± 0.02	138.24 ± 0.80	3.44 ± 0.12	0.98 ± 0.08
R17	6.4 ± 0.64	0.30 ± 0.02	110.93 ± 1.55	6.80 ± 0.04	66.60 ± 0.72	11.26 ± 0.24	1.12 ± 0.07
R18	10.42 ± 0.56	0.59 ± 0.01	134.18 ± 0.91	6.72 ± 0.04	118.4 ± 0.26	4.56 ± 0.25	0.98 ± 0.08
R19	6.84 ± 0.40	0.28 ± 0.01	112.23 ± 0.91	6.82 ± 0.02	66.40 ± 0.48	10.22 ± 0.26	1.12 ± 0.07
R20	15.64 ± 0.46	0.78 ± 0.02	154.53 ± 0.80	6.72 ± 0.02	124.2 ± 0.99	3.56 ± 0.25	0.98 ± 0.72
R21	6.54 ± 0.68	0.23 ± 0.01	111.32 ± 0.58	6.82 ± 0.04	74.60 ± 0.72	10.12 ± 0.22	1.74 ± 0.10
R22	5.76 ± 0.50	0.25 ± 0.01	101.37 ± 0.80	6.78 ± 0.02	64.60 ± 0.72	11.32 ± 0.26	1.06 ± 0.06
R23	5.89 ± 0.40	0.31 ± 0.01	101.17 ± 1.70	6.78 ± 0.04	68.24 ± 0.72	11.64 ± 0.12	1.21 ± 0.06
R24	5.76 ± 0.56	0.26 ± 0.02	102.35 ± 1.10	6.80 ± 0.04	66.54 ± 0.72	11.48 ± 0.16	1.84 ± 0.08
R25	5.84 ± 0.46	0.26 ± 0.02	102.31 ± 1.10	6.74 ± 0.02	67.66 ± 0.72	11.22 ± 0.18	1.14 ± 0.20
R26	12.64 ± 0.36	0.74 ± 0.02	144.34 ± 1.10	6.68 ± 0.04	114.2 ± 0.99	4.56 ± 0.22	0.98 ± 0.03
R27	6.84 ± 0.40	0.28 ± 0.02	110.42 ± 1.60	6.72 ± 0.06	68.24 ± 0.72	11.12 ± 0.40	1.88 ± 0.02

All values are expressed as mean ± *SD, n=3;* %S= Swelling percentage PMA = Percentage Moisture Absorption; PML = Percentage Moisture Loss

Table 6: Comparative *in vivo* pharmacokinetic studies data between Carvedilol treatment groups

Parameter	Carvedilol in CMC (0.5mg/PO)	Carvil (0.5mg) PO	Carvidilol buccal film (R20)	Carvidilol buccal tablet (BT7)
Tmax (h)	2	2	4	8
Cmax (µg/mL)	0.321	0.441	0.261	0.288
AUC $_{0-\alpha}$ (µg/mL/h)	110.14	146.56	1864.46	841.02
MRT 0-α (h)	4	4	28	16
F rel= $\frac{(AUC)_{drug}.(Dose)_{std}}{(AUC)_{std}.(Dose)_{drug}}$		Bioavailability enhanced by 1.32%	Bioavailability enhanced by 12.77%	Bioavailability enhanced by 5.76%

Note: Increase in $AUC_{0-\infty}$; MRT; Tmax; Decrease in Cmax in buccal flim patch shows better enhancement of bioavailability than other two dosage form. [Tmax (h): Time to reach peak plasma concentration; Cmax (µg/mL): Peak plasma concentration.; AUC 0-α (µg/mL/h): Area under the plasma concentration-time curve from 0 to the last measurable time point; MRT 0-α (h): Mean Residence Time from 0 to the last measurable time point; F rel: Relative Bioavailability, calculated as the ratio of AUC (drug) / Dose (std) divided by AUC (std) / Dose (drug)].

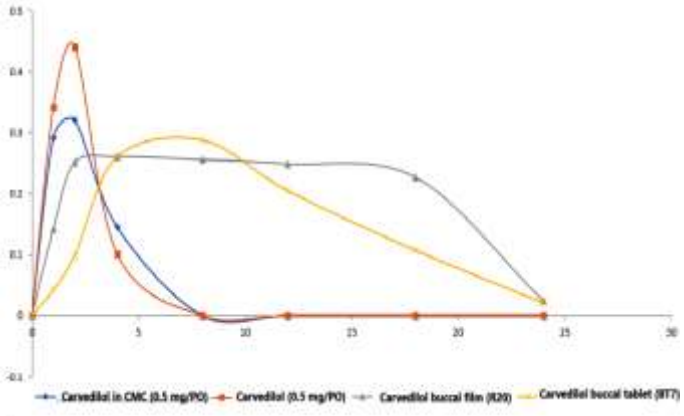

Figure 4: Graph of Comparative *in vivo* Pharmacokinetic study data between Carvedilol treatment groups

As a result, it was established that the R20 buccal film followed zero order kinetics, which release the same amount of drug in a controlled and predetermined manner at unit time intervals. It was the ideal formulation for the drug's release in order to accomplish the required pharmacological impact while avoiding side effects. When the drug release pattern was fitted to Higuchi, the regression r2 value was 0.936, indicating that the patch's drug release pattern followed the diffusion mechanism. The drug release exponent value (n) for R20 formulation was found to be 0.564, which was within the range of n = 0.45-0.89, based on Peppas equation fittings.

Pharmacokinetic Studies of various Carvedilol formulations
HPLC results for Quantification of Carvedilol in plasma
To determine the unknown plasma drug concentration of Carvedilol a calibration curve was designed by using different concentration of Carvedilol. The linearity for calibration curve was determined by plotting the peak area and nominal concentration of Carvedilol. For linearity study eight different concentration of Carvedilol were analyzed (04, 8, 12, 16, 20, 24, 28, 32 µg/mL). The peak area response was found to be linear over the concentration range studied. The coefficient of correlation 'r^2' was found to be 0.9975.
The HPLC method by interpolation technique has been successfully used to determine the pharmacokinetic data from unknown plasma drug concentration followed by single dose administration of Carvedilol (Carvil®), Carvedilol buccal film (R20) Carvedilol buccal tablet (BT7). From the peak area of injected sample the unknown concentration was determined. The mean plasma concentration of Carvedilol vs. function of time has been plotted in Figure 4 and the Comparative studies on *in vivo* plasma drug concentration profile between marketed Carvedilol tablet (Carvil®); Carvedilol buccal film (R20); Carvedilol buccal tablet (BT7) was tabulated in Table 6. It was observed that Carvedilol buccal film (R20) and Carvedilol buccal tablet (BT7) controlled the release as well as pharmacokinetic parameters when compared to the Carvedilol marketed formulation. There was a significant difference with $p < 0.05$ between the pharmacokinetic parameters of marketed Carvedilol, Carvedilol buccal film (R20); Carvedilol buccal tablet (BT7) with T_{max} of 2hrs, 4hrs and 8hrs; maximum peak plasma concentration (C_{max}) of 0.441µg/mL, 0.261µg/mL and 0.288µg/mL respectively. Area Under Curve ($AUC_{0-\alpha}$) was found to be 146.56 µg/mL/h, 1864.46µg/mL.h, 841.02µg/mL.h, Mean Residence Time of drug $MRT_{0-\alpha}$ was found to be 4hrs, 28hrs and 16hrs respectively. pharmacokinetic data was analysed by PK Solver Excel sheet. From the *in vivo* pharmacokinetic data it was concluded that increase in $AUC_{0-\infty}$, T_{max}, MRT with decrease in C_{max} in NLC and buccal flim when compared to marketed available Carvil tablet. On calculating the relative bioavailability by keeping marketed formulation as standard, it has been confirmed that carvedilol buccal flim shows enhancement of bioavailability of about 12.77 % than other two formulations.

Conclusion

The study emphasizes the significance of rigorous formulation optimization and the use of QbD principles in improving drug delivery systems. The results showed R20 as the best formulation, with a 3 mL natural polymer solution concentration (Lime basil mucilage), 0.75% plasticizer concentration, and a specific amount of permeation enhancer. This formulation had favorable features such as 96.9% 2.4 drug content, 97.4%±2.4 drug release after 24 hours, and a folding endurance of 422±12. These optimized parameters for the manufacturing of buccal films have been established, for further study and development of buccal drug delivery systems.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Vanessa H, Vidya S, Katrusha H, Danica Vidović Juras, Martin Greenberg. New developments and opportunities in oral mucosal drug delivery for local and systemic disease. Adv Drug Deliv Rev.2012;64(01):16-28.
2. Hoogstraate JAH, Wertz PW. Drug delivery via the buccal the buccal mucosa. J Pharm Sci Tech Today. 1998;1(01):309-316.
3. Mathias NR and Hussain AM. Non-invasive systemic drug delivery: develop ability considerations for alternate routes of administration. J Pharm Sci 2010; 99(01):1-20.
4. Shojaei Amir H. Buccal Mucosa as a route for systemic drug delivery. J Pharm Sci. 1998; 1(01):15-30.
5. Flávia Chiva Carvalho, Marcos Luciano Bruschi, Raul Cesar Evangelista, Maria Palmira Daflon Gremião. Mucoadhesive drug delivery systems. Braz J Pharm Sci. 2010; 46: 1-18.
6. Chinna Reddy PKS, Chaitanya, Madhusudan Rao Y. A review on bioadhesive buccal drug delivery systems: current status of formulation and evaluation methods. Daru. 2011; 19(06):385–403.
7. Satishbabu BK, Srinivasan BP. Preparation and evaluation of buccoadhesive films of carvedilol. Indian J Pharm Sci. 2008;70(02):175-179.
8. Amanpreet Kaur, Gurpreet Kaur. Mucoadhesive buccal patches based on interpolymer complexes of chitosan pectin for delivery of Carvedilol. Saudi Pharm J. 2012; 20(01):21-27.
9. Yamsani VV, Gannu R, Kolli C, Rao MEB, Yamsani MR. Development and *in vitro* evaluation of buccoadhesive carvedilol tablets. J Acta Pharm. 2007;57(2):185-197.
10. Abd-Elbary A, AMA Makky, MI Tadros and AA Alaa-eldin. Development and *in v*itro evaluation of mucoadhesive bilayer buccal tablets of carvedilol. Int J Pharm Sci. 2015; 7(01):172-176.
11. Khazaei N, Esmaiili M, Djomeh ZE, Ghasemlou M, Jouki M. Characterization of new biodegradable edible film made from basil seed (Ocimum basilicum L.) gum. Carbohydrate polymers. 2014;15(102):199-206.
12. Trastullo R, Abruzzo A, Saladini B. Design and evaluation of buccal films as paediatric dosage form for transmucosal

delivery of ondansetron. Eur J Pharm Biopharm. 2016; 105:115–121.
13. Dipak Malpure rajaram, Sharada Deore Laxman. Buccal mucoadhesive films: A review; Syst Rev Pharm. 2017; 8(01): 31-38.
14. Gamal M El-Maghraby, Mona M Abdelzaher. Formulation and evaluation of simvastatin buccal film. J Appl Pharm Sci. 2015;5(04):70-77.
15. Satishbabu BK, Srinivasan BP. Preparation and evaluation of buccoadhesive films of atenolol. Indian J Pharm Sci. 2008;70(02):175-179.
16. Abouhussein, El-bary, Shalaby and El-Nabarawi. Chitosan mucoadhesive buccal films: effect of different casting solvents on their physicochemical properties. Int J Pharm Sci. 2016; 8(09):206-213.
17. Singh B, Chakkal S K, Ahuja N. Formulation and Optimization of Controlled Release Mucoadhesive Tablets of Atenolol Using Response Surface Methodology. JAAPS Pharm Sci Tech. 2006; 7(01):19-22
18. Vishal V, Bilaskar, Indrajeet S Patil, Omkar A patil, Girishchandra R Mandke, Shrinivas K Mohite. Design, development and optimization of pulsatile drug delivery of antihypertensive drug. International research journal of pharmaceutical and biosciences. 2018; 4(06):12-19.
19. Shrivastava AR, Ursekar B, Kapadia CJ. Design, optimization. preparation and evaluation of dispersion granules of valsartan and formulation into tablets. Curr Drug Deliv. 2009;6(01):28‑37.
20. Peh KK, Wong CF. Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. J Pharm Sci. 1999; 2(02):53-61.
21. Perumal V A, Lutchman D, Mackraj I, Govender T. Formulation of monolayered films with drug and polymers of opposing solubilities, Inter Jou of Pharmaceutics 2008;358 (01):184–191.
22. Joydip Kundu, Chinmoy Patra, Kundu S C. Design, fabrication and characterization of silk fibroin-HPMC-PEG blended films as vehicle for transmucosal delivery, Materials Science and Engineering.2008;28(08):1376–1380.
23. Alanazi F K, Abdel Rahman A, Mahrous G M, Alsarra I A. Formulation and physicochemical characterization of buccoadhesive films containing ketorolac, J Drug Del Sci Tech. 2007; 17(01) :1-10.
24. Noha Adel Nafee, Nabila Ahmed Boraie, Fatma Ahmed Ismail, Lobna Mohamed Mortada. Design and characterization of mucoadhesive buccal patches containing Cetylpyridinium chloride, Acta Pharm. 2003; 53(03):199–212.
25. Myung Kwan Chun, Byoung Tae Kwak, Hoo Kyun Choi. Preparation of Buccal Patch Composed of Carbopol, Poloxamer and Hydroxy propyl methyl cellulose, Arch Pharm Res. 2003; 26(11):973-978.
26. Rajesh Singh Patel, Poddar S. Development and characterization of mucoadhesive buccal Patches of Salbutamol sulphate, Current Drug Delivery. 2009;6(01):140-144.
27. Roy S, Pal K, Anis A, Pramanik K, Prabhakar B. Polymers in Mucoadhesive Drug Delivery System: A Brief Note, Designed monomers and polymers. 2009; 12:483-495.
28. Rinku Khurana, Alka Ahuja, Roop K Khar. Development and evaluation of mucoadhesive films of miconazole nitrate, Indian J Pharm Sci.2000; 62(06):447-453.
29. Kharenko E A, Larionova N I, Demina N B. Mucoadhesive drug delivery systems: quantitative assessment of interaction between synthetic and natural polymer films and mucosa, Pharmaceutical Chemistry Journal.2008; 42(07):392-399.
30. Kashappa Goud HD, Pramod Kumar TM. Preparation and Evaluation of a Novel Buccal Adhesive System, AAPS PharmSciTech. 2004; 5(03) :1-9.
31. Balamurugan K, Pandit J K, Choudary P K, Balasubramaniyam J. Systemic absorption of Propranolol Hydrochloride from buccoadhesive films, Indian J Pharm Sci. 2001; 63(06) :473-480.
32. Ellen Hagesaether, Marianne Hiorth, Sverre Arne Sande. Mucoadhesion and drug permeability of free mixed films of pectin and chitosan: An *in vitro* and *ex vivo* study, European Journal of Pharmaceutics and Biopharmaceutics.2009; 71(2):325–331.
33. Supriya S. Shidhaye, Nilesh S. Saindane, Sagar Sutar, Vilasrao Kadam. Mucoadhesive Bilayered Patches for Administration of Sumatriptan Succinate, AAPS PharmSciTech. 2008; 9(3):909-916.
34. Shaila Lewis, G.Subramanian,S Pandey, N Udupa. Design, evaluation and pharmacokinetic study of mucoadhesive buccal tablets of nicotine for smoking cessation, Indian J Pharm Sci.2006; 68(06):829-831.
35. Koland M, Charyulu RN, Vijayanarayana K, Prabhu P. *In vitro* and *in vivo* evaluation of chitosan buccal films of ondansetron hydrochloride. Int J Pharm Investig. 2011; 1(3):164-71.
36. Zaman M, Hanif M, Shaheryar ZA. Development of Tizanidine HCl-Meloxicam loaded mucoadhesive buccal films: *In vitro* and *in vivo* evaluation. PLoS One. 2018; 22; 13(3):e0194410.