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DESIGN, DEVELOPMENT, AND OPTIMIZATION OF MUCOADHESIVE BUCCAL FILMS OF GANAXOLONE FOR ENHANCED BIOAVAILABILITY

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ABSTRACT
Background: CDD disorder affects children mainly during their first three months of life. The buccal route offers advantages over oral administration for ganaxolone by avoiding first-pass metabolism and providing direct systemic absorption. This study aimed to formulate and characterise mucoadhesive buccal films of ganaxolone to increase its bioavailability. **Methods:** Mucoadhesive buccal films were prepared using a solvent casting technique employing HPMC K4M and Moringa gum as polymers. The formulation was optimized using a 3^2-factorial design, where polymer concentrations were varied systematically to achieve optimal film properties. Nine batches (OF1-OF9) were formulated and evaluated for various physicochemical parameters, mucoadhesive strength, percentage drug content, goat buccal mucosa permeation study, and stability analysis. **Results:** Based on the findings, the OF8 batch containing optimal polymer ratio (250mg HPMC K4M and 60mg Moringa gum) emerged as the superior formulation with 94.45±0.34% drug content, 15.37±0.58 N/mm² tensile strength, and 7.8±0.57 N mucoadhesive strength. Permeation studies consequently confirmed 96.37% of the drug at 8 hours with a 13.63 µg /cm² /h permeation rate. There was no evidence of drug-excipient interaction in FTIR and DSC analysis. The formulation was set to be stable for 6 months at accelerated conditions (40±2°C, 75±5% RH) with an average tensile strength above 15 N/mm² and an average *ex-vivo* drug permeation of 93%. **Conclusion:** This optimized buccal film formulation demonstrates promising potential for clinical application in CDD treatment by offering enhanced bioavailability, controlled release, and patient-friendly administration, which is particularly beneficial for pediatric patients.

INTRODUCTION

CDKL5 deficiency disorder results from genetic mutations in the CDKL5 gene that produce a brain development protein [1]. The disorder affects children mainly during their first three months of life. [2]. The intense seizures that affect CDD patients occur often and do not improve with standard seizure medications, making life difficult for both patients and their families [3]. Studies show CDD affects one in every 40,000 to 60,000 newborns worldwide, and female infants are more likely to develop this condition because the CDD gene resides on their X

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chromosome [4]. Besides seizures, CDD patients experience delayed development and movement problems, which make their care harder to manage [5]. While modern genetic tests have helped doctors detect CDD, the limitations of current therapeutic options necessitate the development of novel drug delivery systems that can improve treatment outcomes [6].

Ganaxolone shows excellent potential as a new treatment for controlling seizures in Carney complex DDS patients [7]. The drug acts as a GABA_A receptor enhancer to increase GABA's calming effect throughout the central nervous system [8]. Using this approach, the treatment helps reestablish normal neuronal function and decreases the excess electrical activity seen in seizure conditions. Clinical studies have demonstrated that ganaxolone exhibits superior efficacy compared to conventional anticonvulsants such as phenobarbital and benzodiazepines, with a 30-40% greater reduction in seizure frequency [9]. Medical scientists altered ganaxolone from natural allopregnanolone to increase its absorption when taken by mouth and make it stable for extended medical treatments. [10]. Research shows that ganaxolone works better than regular seizure medication in managing epilepsy and presents fewer safety risks. Ganaxolone moves through the brain barrier to control GABA activity, which points to its promise as a primary treatment choice for CDD and other treatment-resistant types of epilepsy [11]. Buccal drug delivery brings new benefits to medical treatments by improving the efficiency of how medicine enters the body when compared to standard methods [12]. This route is particularly advantageous for ganaxolone delivery as it bypasses hepatic first-pass metabolism, significantly reducing oral bioavailability [13]. The high blood vessel density in the oral mucosa enables drugs to move quickly into the blood system while avoiding breakdown in the liver [14]. This method enhances drug absorption and maintains steady medication levels in the blood. Studies have shown that buccal delivery can enhance drug bioavailability by up to 60% compared to oral administration [15]. Buccal films are excellent delivery devices because their thin, flexible polymer structure works well with ganaxolone treatment [16]. These films stick to the oral mucous membranes and control how the medicine enters the system over time [17]. The mucoadhesive properties of these films can significantly enhance the residence time and permeation of ganaxolone, potentially improving its bioavailability by 2-3-fold compared to oral administration [18]. These films help patients who cannot swallow regular medications, such as CDD patients, because they are easy to use and do not require invasive procedures. Buccal films make ganaxolone administration more reliable because they consistently improve patient adherence while delivering exact doses [16]. The primary objective of this research is to design, develop, and optimise mucoadhesive buccal films for the delivery of ganaxolone to enhance its therapeutic efficacy in managing seizures associated with CDD. This study aims to evaluate the formulation parameters, optimize the drug release profile, and assess the films' mucoadhesive properties to improve bioavailability and patient compliance.

MATERIALS AND METHODS

Materials

Ganaxolone was purchased from the supplier Sciquaint Innovations OPC Private Limited, Pune, India. Research Lab Fine Chem Industries, Mumbai, supplied Hydroxypropyl methylcellulose K4M, and Moringa gum was purchased from Indianjudibhuti, Delhi. Sorbitol and ethanol were purchased from Merck Limited, Mumbai. Every chemical and reagent for this study met analytical grade requirements.

Methods

Calibration Curve of Ganaxolone

Methanol was chosen as the solvent to investigate the spectrum properties of ganaxolone. 10 mg of ganaxolone was added to a 100 ml (100 µg/ml) calibrated volumetric flask, dissolved, and topped off with methanol. Ganaxolone (100 µg/ml) stock solution was prepared in methanol [19]. To prepare working standard solutions of various concentrations (5-30 µg/ml), a series of 10 ml calibrated volumetric flasks was filled with methanol. This was done by diluting the stock solution of 100 µg/ml, then removing various aliquots (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml) from the standard solution. Each solution's UV absorbance (UV 1900 Shimadzu spectrophotometer, Japan) was determined at 247nm [20].

Determination of Solubility

The solubility of the ganaxolone was determined in water, ethanol, phosphate buffer (pH 6.8), and DMSO, in which 1000 mg of the drug was dissolved in 50 mL of each solvent separately in two hundred ml standard volumetric flask which was closed and put in an orbital shaking water bath maintained at 50 rpm with 37 ± 0.5°C temperature for 48 hrs [21]. Subsequently, the samples were filtered, adequately diluted with the same solvent, and scanned for absorbance at the 247 λ_{max} of each solvent using

a UV-visible spectrophotometer. The absorbance values were then used to calculate the drug concentration in the respective solvent using a standard curve of the ganaxolone [22].

Differential Scanning Calorimetric (DSC) Analysis

Differential scanning calorimetry (DSC) measurements of the pure drug were performed using a Perkin-Elmer Pyris-1 DSC (Osaka, Japan). During sample preparation, samples were first dried using preheating. A portion of the sample size between 3–7 mg was accurately weighed and introduced into a hermetically sealed 40µ Al pan analytical weight used with alpha alumina powder [23]. The analysis was carried out under a temperature scale of 50°C and 300°C in increments of 20°C per minute. The experiment was performed under a nitrogen gas flow rate of 20 mL/min. Infrared Thermograms were obtained to determine the positions of the exotherm peaks; comparison with standard spectra allowed detection of changes or shifts to other positions [24].

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy of the pure drug was analysed using a Fourier Transform Infrared (FTIR)- 8400S spectrophotometer procured from Shimadzu, Japan. The sample was finely ground with potassium bromide (KBr) powder in a 1:100 weight ratio using a mortar and pestle. This was followed by compressing the mixture utilizing a hydraulic press to turn it into a pellet. with a pressure of 15 tons for 1 minute. The pressure was gradually relieved to recover the formed pellet. The pellet was loaded into the sample holder, and the spectral scanning was performed within the region of 4000–400 cm^{-1}, with 4 cm^{-1} steps and a scan rate of 2 mm/sec. The obtained spectra allowed for identifying functional groups present in the substance and evaluating the structural alterations of the drug under study [25].

Experimental Design

A 3^2-factorial design was selected over other experimental designs due to its efficiency in optimizing two factors at three levels with a minimal number of experimental runs, while still allowing for the detection of quadratic effects. This design is particularly suitable for pharmaceutical formulation optimization, where the relationship between factors and responses is expected to be non-linear but not highly complex. The Hydroxypropyl Methylcellulose K4M (A) and Moringa gum (B) are independent variables. The dependent variables that were measured in this study encompassed mucoadhesive strength (N) (R1), Tensile strength (N/mm^2) (R2), and *Ex vivo* drug permeation (%) (R3). All experimental design and data analysis were conducted using Design-Expert software, version 13.0 (Stat-Ease). The findings of the experiments are presented in Tables 1 and 2 [26]. The following polynomial equation was used to analyze the responses:

$$\mathbf{Y = \beta 0 + \beta 1A + \beta 2B + \beta 3AB + \beta 4A2 + \beta 5B2}$$

Y is the dependent variable, β0 is the arithmetic mean response, and β1 to β5 are the regression coefficients. A and B represent the main effects; AB is the interaction between factors; A2 and B2 are the quadratic effects.

Table 1: 3^2 Factorial Design showing independent factors and Levels.

Independent Variables				
Label	**Factors**	**Level (mg)**		
		Low (-)	**Medium**	**High (+)**
A	HPMC K4M	150	250	350
B	Moringa gum	20	40	60
Dependant Variables				
Y_1	Mucoadhesive strength (N)			
Y_2	Tensile strength (N/mm^2)			
Y_3	Ex vivo drug permeation study at 8 h (%)			

Table 2: Factors, levels, and responses taken in 3^2 complete factorial designs for Mucoadhesive buccal film.

F. Code	(A)	(B)
F1	-1	-1
F2	0	-1
F3	+1	-1
F4	-1	0
F5	0	0
F6	+1	0
F7	-1	+1
F8	0	+1
F9	+1	+1

"-" & "+" indicates lower and higher concentration respectively

Formulation of mucoadhesive buccal film

The mucoadhesive buccal films of ganaxolone were formulated using a solvent-casting technique. This procedure was repeated thrice, and Ganaxolone was accurately weighed and dissolved in ethanol (Merck, Mumbai, India) to ensure the drug's complete solubility. Hydroxypropyl methylcellulose (HPMC K4M) and moringa gum were dissolved in distilled water using a magnetic

stirrer (IKA C-MAG HS7, Germany) under constant stirring until a homogenous and viscous polymeric solution was obtained. Sorbitol was added as a plasticiser, giving the desired flexibility and mechanical strength characteristics. Ethanol solution of ganaxolone was slowly added to the polymeric solution under high shear mixing using a mechanical stirrer (REMI RQ-127 A/D, India) at a speed of 500 rpm for 30 minutes to obtain a homogeneously dispersed drug. The mixture was stirred at 500 rpm for 30 minutes, as this speed was determined through preliminary studies to provide optimal mixing while preventing excessive air incorporation and polymer degradation. Sonication at 37 kHz for 15 minutes (Ultrasonic Cleaner, Elmasonic E60H, Germany) was selected based on optimization studies showing complete air bubble removal without affecting polymer integrity. The final volume of the formulation was made up to 100 mL with distilled water before drying [27]. The drying temperature of 45-50°C was chosen as it provided complete solvent removal within 24 hours while preventing thermal degradation of ganaxolone and maintaining optimal film flexibility. This temperature range was determined through preliminary drug and polymer stability studies.

Table 3: Preparation of mucoadhesive buccal film batches using 3^2 factorial designs

Ingredients	OF1	OF2	OF3	OF4	OF5	OF6	OF7	OF8	OF9
Ganaxolone (gm)	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
HPMC K4 M (mg)	150	250	350	150	250	350	150	250	350
Moringa gum (mg)	20	20	20	40	40	40	60	60	60
Sorbitol (%v/v)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ethanol (%w/v)	10	10	10	10	10	10	10	10	10
Water (q.s to 100 ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Characterization of Mucoadhesive Ganaxolone Buccal Films

Surface pH Determination

To measure the surface pH of each buccal film, it was placed in a small beaker containing 5 mL of distilled water, and the surface pH was monitored using a calibrated Digital pH meter (Labtronics, LT-50, India). The experiment was done thrice, and the average of the pH was determined to increase the reliability of the results [28].

Ex Vivo Adhesion Time

The adhesion time of each of the films was determined by fixing the film on a moist surface that resembled the buccal mucosa to determine the adhesive properties of the films. The duration taken before the film was detached from the surface was noted manually. This parameter also measures the ability of the film to retain its characteristics during the application. [29].

Film Weight and Thickness

Uniformity in the weight and thickness of buccal films is essential for the homogeneity of drug and excipient distribution in the film to prevent inconsistent dosing. Portions of the film equivalent to 2cm x 2cm were excised and weighed on a separate balance. The average weight of three samples was recorded. The film thickness was taken at five positions, and an average film thickness was obtained using a digital vernier calliper to ensure the evenness of the thickness [28].

Tensile Strength and Extensibility

The mechanical properties of the buccal films, such as tensile stress and strain, were measured to determine the film's ability to withstand stress during its application. The texture analyser connected with tensile grips was used to mount the film on cards, and stress was applied to the film at a controlled rate of 0.5mm/s.

The force needed to tear the film, known as the tensile strength, and the ability of the film to stretch before breaking, measured in terms of the elongation at break, were measured and compared [30].

Swelling Index

The swelling characteristic of the mucoadhesive film is essential to increase hydrophilicity and improve the interaction between the polymers and the buccal mucosa. 0.1 gram of pre-weighed film (2cm x 2cm) was equilibrated in a phosphate buffer solution of pH 6.8 for a specific duration.

The film was then rinsed with distilled water and gently blotted to remove any excess buffer, after which the weight was again

taken. The swelling index was determined by using the following formula [30]:

$$SI\ (\%) = \frac{Wt\ of\ hydrated\ film - Wt\ of\ dry\ film}{Wt\ of\ hydrated\ film} \times 100$$

Folding Endurance

Folding endurance is the mechanical aspect of the film, which is its ability to withstand the stress of handling and use. This property was measured using an endpoint conversion, where a film was folded at the same spot several times until cracks or breaks were observed to develop. The number of folds required to cause visible damage was noted for three samples, and the average number of folds obtained was calculated [31].

Mucoadhesive Strength

The goat buccal mucosa was collected from the slaughterhouse in Pune and was hydrated in phosphate buffer (pH 6.8) for 15 minutes to ensure consistent surface moisture. The contact time between the film and mucosa was standardized to 60 seconds under a constant force of 0.5 N to ensure reproducible adhesion. These parameters were established through preliminary studies that showed optimal mucoadhesion development while maintaining tissue integrity. The mucoadhesive strength, the force necessary to remove the film from the goat buccal mucosa surface, was measured on a modified weighing pan balance. The goat buccal mucosa was placed on one glass slide and tied on both sides of the assembly during this experiment. The glass slide with the goat buccal mucosa was fixed on one side of the floor below the modified physical balance. The force required to detach the two materials was measured [32].

Drug Content

The film was cut into 2 x 2 cm pieces to allow even dispersion of ganaxolone in a phosphate buffer of pH 6.8, where one piece was dissolved in 100 mL of the buffer. The formaldehyde was removed by filtration, and the solution was read spectrophotometrically at a wavelength of 247nm. The percentage of drug content was determined to ascertain conformity or otherwise [33].

Ex Vivo drug permeation study

The study of ganaxolone transport across goat buccal mucosa was investigated using a Franz diffusion cell. The mucosa was placed between the donor and receptor chambers, while the film was placed directly on the mucosal linings [34]. The receptor compartment comprised phosphate buffer with a pH of 6.8, a temperature of 37±0.5 °C, and a stirring rate of 50 rpm. Samples were withdrawn at different time intervals, 1 to 8 hours. The UV absorbance of samples was taken at 247nm using a UV spectrophotometer (UV 1900 Shimadzu spectrophotometer, Japan). A plot of time versus percentage drug permeation was drawn.

Stability studies

The stability tests were performed on the optimised formulation as per the guidelines of the International Conference on Harmonization (ICH). A 2x2 cm² film was subjected to a butter paper wrapping followed by an aluminium foil. It was exposed to room conditions of 25±2°C, humidity of 60±5% throughout the 3-month accelerated test at a temperature of 40±2°C and humidity of 75±5% [35,36]. The study team determined mucoadhesive properties, mechanical characteristics, drug content, and drug release rate of oral films prepared for 1, 3, and 6 months of storage.

RESULTS

Results of the Calibration curve of ganaxolone

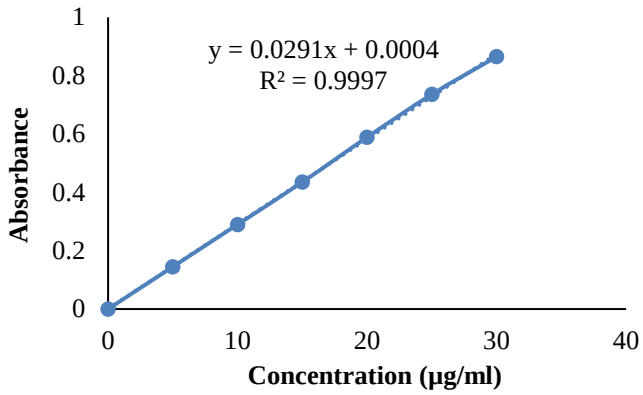

Figure 1: Calibration curve of ganaxolone in methanol

Solubility analysis

Table 4: Results of solubility analysis of ganaxolone

S No.	Solvent	Solubility (mg/ml)	Results
1	Water	0.07±0.005	Practically insoluble
2	Ethanol	6.65±1.42	Slightly soluble
3	Methanol	10.69±0.87	Sparingly soluble
4	Phosphate Buffer pH 6.8	2.59±0.98	Slightly soluble
5	DMSO	20.26±2.83	Sparingly soluble

Values are expressed in mean±SD (n=3)

Results of FTIR analysis

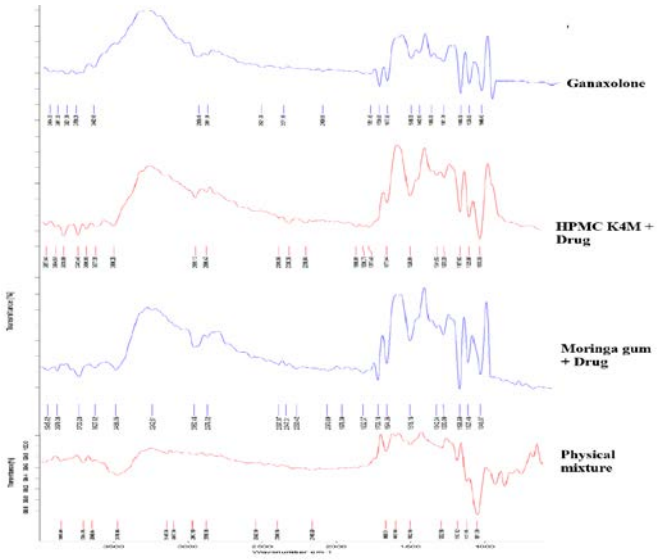

Figure 2: FTIR spectra of Ganaxolone, HPMC K4M + Drug, Moringa gum + Drug and Physical mixture.

Results of DSC Analysis

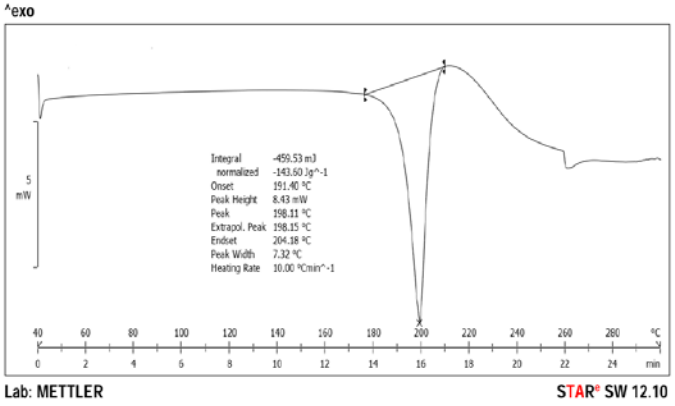

Figure 3: DSC Spectra of ganaxolone

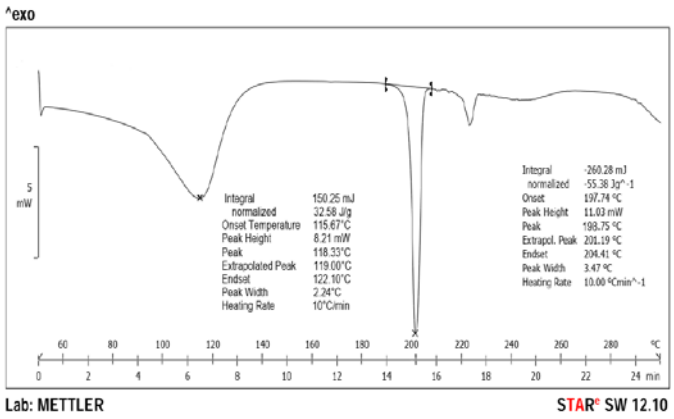

Figure 4: DSC Spectra of Physical mixture (Drug + Excipients)

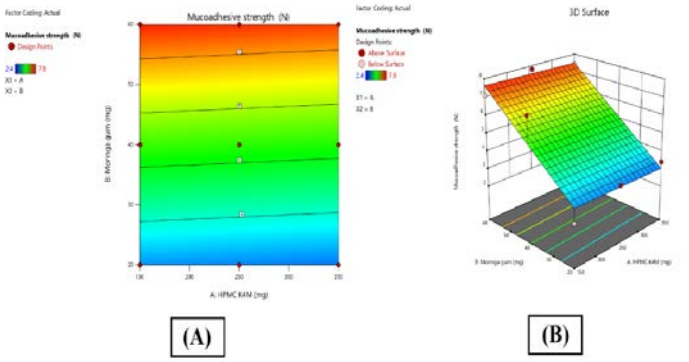

Figure 5: Effect of polymer concentrations on mucoadhesive strength: (A) Contour plot demonstrating increasing mucoadhesive strength (blue to red) with higher Moringa gum concentration; (B) 3D surface plot showing linear relationship between Moringa gum content and mucoadhesive strength, independent of HPMC K4M levels

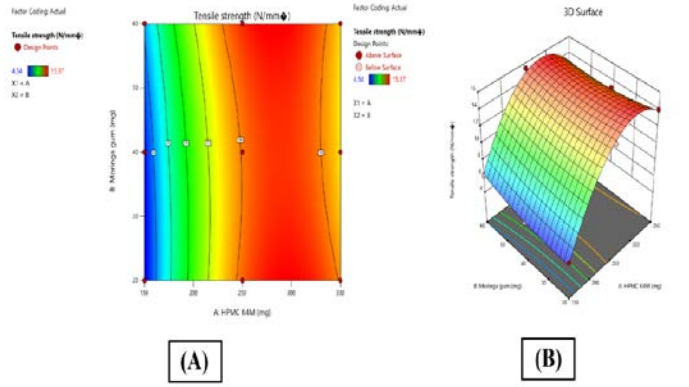

Figure 6: Effect of polymer composition on tensile strength: (A) Contour plot illustrating optimal tensile strength (red region) at moderate-to-high HPMC K4M levels; (B) 3D surface plot revealing quadratic relationship between HPMC K4M concentration and tensile strength

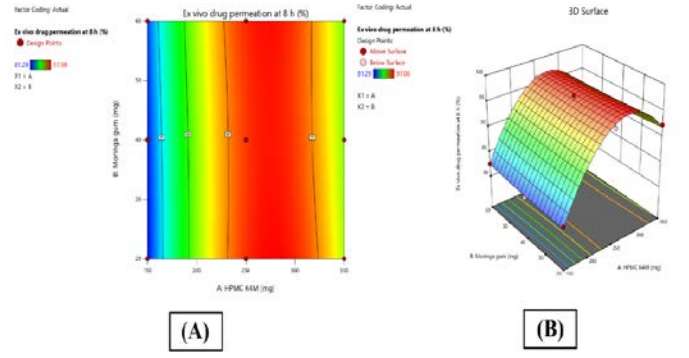

Figure 7: Effect of polymer concentrations on Drug permeation (A) Contour plot showing optimal drug permeation (>95%) in the central region; (B) 3D surface plot demonstrating bell-shaped relationship between HPMC K4M concentration and drug permeation

EVALUATIONS OF MUCOADHESIVE BUCCAL FILM

Table 5: Physicochemical Evaluation of mucoadhesive buccal film (OF1-OF9)

Batch code	Weight variation (mg)	Thickness (mm)	FoldingEndurance	% Moisture Uptake	% moisture Loss
OF1	236.5±2.4	0.14±0.02	> 300	0.14±0.04	0.36±0.01
OF2	223.3±4.7	0.10±0.03	> 300	1.52±0.25	2.82±0.78
OF3	298.7±6.7	0.13±0.02	> 300	1.43±0.18	1.87±0.93
OF4	278.3±3.9	0.14±0.04	> 300	0.39±0.18	1.43±0.09
OF5	316.3±2.9	0.07±0.01	> 300	1.73±0.35	2.39±0.49
OF6	289.4±4.2	0.08±0.01	> 300	1.49±0.05	1.47±0.45
OF7	276.6±8.7	0.07±0.01	> 300	2.57±0.01	1.47±0.39
OF8	287.1±9.4	0.14±0.04	> 300	1.63±0.05	0.83±0.08
OF9	252.9±8.3	0.10±0.02	> 300	0.38±0.03	2.63±0.79

Values are expressed in mean±SD (n=3)

Table 6: Results of tensile strength, drug content and *Ex Vivo* Adhesion Time of mucoadhesive buccal film (OF1-OF9).

Batch code	Tensile strength(N/mm²)	Drug content(%)	Mucoadhesive strength(N)	*Ex Vivo* Adhesion Time(hr)
OF1	4.54±0.85	87.08±0.57	2.4±0.43	5.2±0.03
OF2	13.91±0.23	78.89±0.58	3.2±0.31	5.7±0.02
OF3	14.02±0.92	84.39±0.59	3.4±0.06	5.4±0.01
OF4	4.61±0.24	77.09±0.60	6.9±0.78	5.6±0.05
OF5	14.04±0.74	93.15±0.52	4.7±0.42	5.4±0.08
OF6	13.08±0.67	85.26±0.13	5.1±0.93	5.9±0.03
OF7	6.14±0.35	78.63±0.83	7.1±0.69	6.1±0.07
OF8	15.37±0.58	94.45±0.34	7.8±0.57	6.4±0.05
OF9	12.81±0.34	84.72±0.19	7.4±0.39	6.8±0.09

Values are expressed in mean±SD (n=3)

Optimization of the Concentrations of HPMC K4M and Moringa gum using 3^2 Factorial design.

ANOVA for Linear model for Mucoadhesive strength (Y_1)

Table 7: ANOVA for the Linear model Mucoadhesive strength (Y_1)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	29.52	2	14.76	23.58	0.0014	significant
A-HPMC K4M	0.0417	1	0.0417	0.0665	0.8051	
B-Moringa gum	29.48	1	29.48	47.09	0.0005	
Residual	3.76	6	0.6261			
Cor Total	33.28	8				

The regression equation obtained for Mucoadhesive strength is as follows:

$$Mucoadhesive\ strength = +5.33 - 0.0833 * A + 2.22 * B$$

ANOVA for Quadratic model for Tensile strength (Y_2)

Table 8: ANOVA for the quadratic model Tensile strength (Y_2)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	159.10	5	31.82	162.50	0.0008	significant
A-HPMC K4M	101.02	1	101.02	515.92	0.0002	
B-Moringa gum	0.5704	1	0.5704	2.91	0.1864	
AB	1.97	1	1.97	10.08	0.0503	
A^2	54.92	1	54.92	280.45	0.0005	

B^2	0.6161	1	0.6161	3.15	0.1742	
Residual	0.5874	3	0.1958			
Cor Total	159.69	8				

The regression equation obtained for Tensile strength is as follows:

$$\textbf{Tensile strength (Y2)} = +273.01 + 6.25 * A + 277.20 * B + 3.16 * AB - 0.0767A^2 + 70.58 * B^2$$

ANOVA for Quadratic model for ex vivo drug permeation study at 8 h (Y_3)

Table 9: ANOVA for the quadratic model *ex vivo* drug permeation study at 8 h (Y_3)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	324.97	5	64.99	88.43	0.0019	significant
A-HPMC K4M	136.71	1	136.71	186.00	0.0009	
B-Moringa gum	0.0131	1	0.0131	0.0178	0.9024	
AB	1.02	1	1.02	1.39	0.3237	
A^2	187.15	1	187.15	254.63	0.0005	
B^2	0.0854	1	0.0854	0.1162	0.7556	
Residual	2.20	3	0.7350			
Cor Total	327.18	8				

The regression equation obtained for *ex vivo* drug permeation study at 8 h (Y_3) is as follows:

$$\textit{Ex vivo drug permeation study at 8 h } (Y3) = +96.19 + 4.77 * A + 0.0467 * B - 0.5050 * AB - 9.67A^2 + 0.2067 * B^2$$

Table 10. Summary of the quadratic model results for regression analysis of responses R1 and R2.

Quadratic Model	R^2	Adjusted R^2	Predicted R^2	SD	% CV
Response (Y_1)	0.8871	0.8495	0.7492	0.79	14.84
Response (Y_2)	0.9963	0.9902	0.9552	0.44	4.04
Response (Y_3)	0.9933	0.9820	0.9438	0.85	0.95

Validation of statistical model.

Table 11: The predicted and experimental values of response variables and relative error (RE).

F. Code	Composition	Actual (mg)	Response	Predicted value	Experimental value	RE (%)
OF8	HPMC K4M	250	Mucoadhesive strength (N)	7.5	7.8	3.92
	Moringa Gum	60				
OF8	HPMC K4M	250	Tensile strength (N/mm^2)	15.41	15.37	0.26
	Moringa Gum	60				
OF8	HPMC K4M	250	Ex vivo drug permeation study at 8 h	96.91	96.37	0.56
	Moringa Gum	60				

Ex-vivo drug permeation studies from goat buccal mucosa

The results of *Ex-vivo* drug permeation studies are shown in Figure 8.

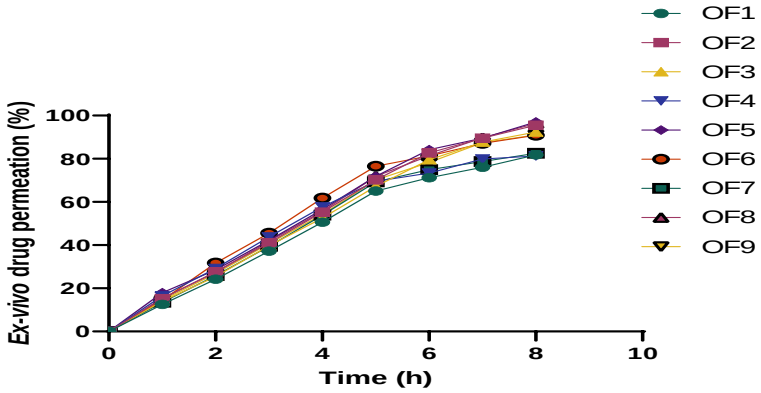

Figure 8: *Ex-vivo* drug permeation studies of the mucoadhesive buccal film (OF1-OF9)

Flux and Kp of mucoadhesive buccal film

Table 12: Flux and Kp of mucoadhesive buccal film

Batch	Flux (µg/cm²/h)	Kp (cm/h)
OF1	12.27	12.27
OF2	13.69	13.69
OF3	13.29	13.29
OF4	11.99	11.99
OF5	13.59	13.59
OF6	13.85	13.85
OF7	12.90	12.90
OF8	13.63	13.63
OF9	13.20	13.20

Stability Study

Table 13: Stability Studies results of optimised Batch (OF8) of mucoadhesive buccal film

Stability conditions	Sampling time	Tensile strength (N/mm²)	Thickness (mm)	Folding Endurance (no. of folds)	Mucoadhesive strength (N)	*Ex vivo* drug permeations (%)
Accelerated condition (40 ± 2°C and 75 ± 5% RH)	Initial (0)	15.94±0.54	0.14±0.06	>300	7.8±0.43	94.78±0.56
	1 Month	15.89±0.67	0.14±0.03	>300	7.8±0.12	94.72±0.87
	3 Months	15.57±0.12	0.14±0.09	>300	7.7±0.26	93.67±0.98
	6 Months	15.32±0.37	0.14±0.06	>300	7.7±0.18	93.23±0.83

Values are expressed in mean±SD (n=3)

DISCUSSION

The calibration curve of ganaxolone in methanol (Figure 4) shows a linear relationship between absorbance and concentration with a high R^2 value, symbolising a reliable method. These findings are consistent with the past literature about analogous reagents and further support the applicability of UV-Vis spectrophotometry in ganaxolone quantification. The usefulness and quality of this method have shown that it can be used for quality assurance and formulation. The solubility (Table 6) proves that ganaxolone is almost insoluble in water, while it is soluble in methanol and DMSO and moderately soluble in ethanol and phosphate buffer 6.8.

The FTIR spectra of ganaxolone, HPMC K4M + ganaxolone, moringa gum + ganaxolone, and physical mixture (Figure 2) show the drug-excipient compatibility. The transmission peak positions of ganaxolone are O-H stretching (3934.33–3642.48 cm^{-1}), C-H stretching (2936.41–2881.91 cm^{-1}), C=O stretching (2109.66–1729.26 cm^{-1}), C=C stretching (1677.90–1518.38 cm^{-1}), C-H bending (1462). These peaks were traceable in the HPMC K4M and moringa gum formulations, suggesting no drug-excipient interaction occurred between ganaxolone and the two excipients. The DSC thermogram of ganaxolone is shown in Figure 3, which illustrates a sharp endothermic effect at 198.11°C, which can be attributed to the melting point of ganaxolone and indicates crystallinity. The physical mixture in Figure 4 shows two peaks at 118.33°C and 198.75°C, indicating that the drug and the excipients partially interact, which could be attributed to the melting of the components of the excipients and slight alterations in the thermal profile of the drug.

The mucoadhesive buccal films of batches OF1-OF9 revealed an appreciable physicochemical profile of batch-to-batch variation in weight average (Table 5), where the weight range was 35.58 – 38.10 mg, the thickness of 0.190 – 0.194 mm, and folding endurance of the buccal films at 7/3 cycles. The folding endurance of all formulations was found to be >300, confirming the formulations' adequate flexibility and mechanical strength. Swelling and shrinkage responses were similarly affected by batch differences, such that OF7 displayed the most significant swelling response of 2.57±0.01% while OF1 had the least swelling response of 0.14 ± 0.04%. These variations indicate that the hydrophilic nature of the formulations is different due to the presence of various excipients. The tensile strength, drug content, and mucoadhesive strength (Table 6) also endorse the efficacy of the film. OF8 had the highest tensile strength (15.37±0.58 N/mm²), drug content (94.45±0.34%), and mucoadhesive strength (7.8 ± 0.57 N), making it a suitable system for buccal delivery. However, OF1 possessed the lowest percentage of the drug (87.08 ± 0.57%) and tensile strength (4.54 ± 0.85 N/ mm²) among the three formulations. These findings indicate that the composition of OF8 offers the best physicochemical and mechanical characteristics for efficient drug delivery. The *ex vivo* adhesion time for nine different

batches (OF1-OF9), revealed a gradual increase in adhesion time. The range of values for OF1 and OF9 was 5.2±0.03 and 6.8±0.09 hours, respectively. A 3^2 factorial design optimised HPMC K4M and moringa gum concentrations on mucoadhesive strength (Y_1). Table 7 presents the ANOVA results, which show that the model effect is statistically significant ($p = 0.0014$); however, the concentration of HPMC K4M (A) does not have a significant effect ($p = 0.8051$), while moringa gum concentration (B) has a very significant effect ($p = 0.0005$) on the mucoadhesive strength (Y_1).

The relationship between mucoadhesive strength and film composition significantly correlates with Moringa gum concentration (F-value=47.09, $p = 0.0005$). The analysis of variance reveals that Moringa gum's influence on mucoadhesive properties is primarily due to its abundant hydroxyl groups forming stronger hydrogen bonds with the mucin glycoproteins. At the optimal concentration of 60 mg, Moringa gum provides enhanced surface wetting properties and optimal polymer chain flexibility, leading to superior mucoadhesion. The contour plot for mucoadhesive strength (Figure 5A) shows concentric regions of increasing strength (2.4 N to 7.8 N) as Moringa gum concentration increases, with the effect size (F-value = 47.09) confirming Moringa gum as the primary determinant.

The characterisation of the buccal films was done using tensile strength, whereby the optimum value was found at three factors using the quadratic model, as shown in Table 8. The analysis of variance (ANOVA) confirms the significance of the model ($p = 0.0008$); also, according to the results of the study, the most significant factor affecting the release of the drug is the concentration of HPMC K4M (A = 0.0002%), while the interaction between HPMC K4M and moringa gum (AB = 0.0503%) [49]. The interaction between moringa gum concentration (B) and HPMC K4M was also not statistically significant ($p = 0.1864$). Higher-order effects like A^2 ($p = 0.0005$) affected tensile strength and suggest curvilinear effects.

The results obtained from Equation 1 show that the independent parameter of HPMC K4M positively influences tensile strength, while the parameter 'AB' establishes the interaction between both components. The tensile strength response (Figure 6) exhibits a more complex relationship, with the high effect size of HPMC K4M (F-value = 515.92) manifesting as a curved surface peaking at moderate-to-high HPMC K4M levels.

The optimal value of the *ex vivo* drug permeation study at 8 h (Y_3) was further predicted using the quadratic model, as shown in Table 9. The analysis of variance, briefly ANOVA, shows that the model is significant at $p = 0.0019$, and HPMC K4M (A) concentration has the highest significance at $p = 0.0009$. The inclusion of moringa gum concentration of (B) level and the interactions between moringa gum and HPMC K4M (AB) were not significant ($p > 0.05$). These general effects, including A^2 ($p = 0.0005$), also had a higher-order impact on drug release non-linearly. Equation 1 shows that the increase in the concentration of HPMC K4M positively influences the release of the drug, while the A^2 confirms that the impact becomes slightly negative with increased concentration. Drug permeation (Figure 7) shows an optimal region at intermediate HPMC K4M concentrations (F-value = 186.00), with the bell-shaped response surface indicating that excessive polymer concentrations may hinder drug release.

The *ex vivo* permeation studies on goat buccal tissue showed a variation in drug permeation in the formulations (Figure 8). The permeation at 8 hours ranged between 81.81% of OF1 and 97.08% of OF5 and OF8, with 96.37%. The drug release kinetics followed the Higuchi model ($R^2 = 0.9933$), demonstrating that diffusion served as the main drug release mechanism. Buccal permeation profiles (Figure 8) reveal two distinct phases, beginning with initial burst drug release during the first two hours, then transitioning to an extended sustained delivery phase lasting 8 hours. The drug release occurs in two stages after an initial surface dissolution of the drug, which then undergoes controlled diffusion through the polymer network. The optimized formulation OF8 released 96.37% of the drug through buccal mucosa permeation during the 8 hours. A stable hydration layer that develops forms a protective barrier, regulating drug diffusion speeds, thereby extending the drug release duration.

The selection of OF8 as optimal formulation occurred due to complete suitability across multiple critical parameters, even though OF6 exhibited a slightly higher flux at 13.85 µg/cm²/h compared to OF8 at 13.63 µg/cm²/h. OF8 was selected as the optimal formulation since it demonstrated enhanced parameters, including superior tensile strength (15.37±0.58 N/mm²) compared to OF6 (13.08±0.67 N/mm²), along with higher drug content uniformity (94.45±0.34% vs 85.26±0.13%) and better mucoadhesive strength (7.8±0.57 N vs 5.1±0.93 N). These better properties in OF8 help overcome its marginally reduced flux

because they ensure stability and therapeutic performance. The combination of HPMC K4M: 250 mg and Moringa gum: 60 mg leads to optimal mechanical properties in OF8. The 250 mg HPMC K4M ratio with 60 mg Moringa gum creates better molecular interweaving between polymer chains, resulting in a strong polymer network. ANOVA results demonstrated that HPMC K4M (F-value = 515.92, p = 0.0002) significantly impacted tensile strength among the tested groups.

Stability studies of the optimised formulation (OF8) were carried out under accelerated conditions (40±2°C and 75±5% RH for 6 months (Table 13). The findings indicated that tensile strength, oxygen permeability, folding endurance, mucoadhesive strength, and *ex vivo* drug permeation did not significantly change. The tensile strength was maintained above 15 N/mm², and the *ex vivo* drug permeation was above 93%, which shows good stability with the developing formulation. Results showed no statistically significant changes ($p>0.05$) in critical parameters over the storage period. In accordance with ICH Q1A(R2) guidelines for accelerated stability testing, all parameters remained well within acceptance criteria. The tensile strength showed minimal variation (3.89% from the initial value), while drug content and mucoadhesive strength variations were 1.63% and 1.28%, respectively, within the ICH limit of ±5%. These results confirm the formulation's stability under accelerated conditions, suggesting suitable stability for long-term storage and clinical application.

Recent pharmacokinetic studies have demonstrated that the 3β-methyl modification of ganaxolone significantly enhances its oral bioavailability from 3% to approximately 15% compared to allopregnanolone. This structural modification improves the drug's stability and absorption characteristics, supporting its development as an orally active neurosteroid. Combining this enhanced bioavailability with our optimized buccal delivery system suggests potential for improved therapeutic outcomes in CDD treatment.

CONCLUSION

The developed buccal film system offers several potential clinical advantages over the current CDD formulations, including ease of administration, particularly advantageous for pediatric CDD patients with swallowing difficulties, and improved patient compliance due to non-invasive administration. The sustained mucoadhesion time reduces dosing frequency and the sustained release and improved bioavailability enhance therapeutic efficacy. Future research should involve in vivo pharmacokinetic studies in animal models, industrial scale up manufacturing, and clinical trial to assess safety and efficacy of this delivery system in CDD patients. Stability studies under various environmental conditions and package configurations will also be necessary for commercialization of this promising drug delivery system.

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NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Onkar Pawar and Rahul Godge contributed in Planning, conceptualization, data collection and paper writing. Ganesh Shinde, Kailas Barde, Akshay Vikhe Contributed in Data collection, review of literature and data interpretation. All authors contributed to the completion of the manuscript.

ABBREVIATIONS

CDD: CDKL5 Deficiency Disorder; CDKL5: Cyclin-Dependent Kinase-Like 5; GABA: Gamma-Aminobutyric Acid; UV: Ultra-Violet; FTIR: Fourier-transform Infrared; DSC: Differential Scanning Calorimetry; HPMC K4M: Hydroxypropyl Methylcellulose K4M; DMSO: Dimethyl Sulfoxide; KBr: Potassium Bromide; ANOVA: Analysis of Variance; UV-Vis: Ultraviolet-Visible; SD: Standard Deviation; RH: Relative Humidity; ICH: International Conference on Harmonisation; OF: Oral Film; Kp: Permeability Coefficient; rpm: Rotations Per Minute; q.s.: Quantum Sufficient.

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