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Pushing Limits: Exploring Torsemide's Potential Through *In-Vitro* Mucoadhesive Buccal Delivery Characterization

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Abstract

This study focuses on developing and evaluating mucoadhesive buccal tablets containing Torsemide, utilizing HPMC K-100, Xanthan Gum, and chitosan polymers at varying concentrations. Physicochemical parameters were well-maintained according to Indian Pharmacopeia standards. Swelling indices ranged from 201.75% to 241.85% over 8 hours, with suitable tablet pH (6.8-6.9) for buccal administration. Mucoadhesive strengths (18.00-27.33 g) varied with polymer concentration. Batch 6 displayed sustained Torsemide release (73.73% at 8 h), supported by optimized swelling index and mucoadhesive strength. Formulation F6, combining Xanthan Gum and Chitosan, emerged as optimal. Dissolution followed zero-order kinetics, fitting the Korsmeyer-Peppas model, indicating a diffusion-based, non-Fickian mechanism. No Torsemide-excipient interactions were observed through Fourier Transform Infrared Spectroscopy. This study successfully designed controlled-release mucoadhesive buccal tablets, influenced by polymer behavior and concentration, advancing drug delivery systems.

Keywords: *Buccal, Chitosan, Mucoadhesive, Discharge, Tablet, Torsemide.*

1. Introduction

Edema is considered by the accumulation of excess fluid within the body's tissues, resulting in swelling. It can manifest in various body parts, with hands, arms, feet, ankles, and legs being common areas of noticeable swelling[1]. The causes of edema can vary and encompass factors such as medication usage, pregnancy, or an underlying health condition, often including congestive heart failure, kidney disease, or liver cirrhosis. Addressing edema typically involves the administration of medications to eliminate surplus fluid and a reduction in dietary salt intake, which can help alleviate the swelling. However, if edema is indicative of an underlying ailment, that specific disease necessitates separate and distinct treatment. It is crucial to recognize that edema might signify a more severe underlying medical issue that requires attention[2].

Edema can stem from various underlying conditions, each contributing to fluid accumulation in distinct ways. In congestive heart failure, the heart's lower chambers lose their efficiency in pumping blood, causing blood to pool in the legs, ankles, and feet, resulting in edema. This condition can also lead to abdominal swelling and, in severe cases, fluid accumulation in the lungs (pulmonary edema), and leading to breathlessness. Cirrhosis of the liver can prompt fluid buildup in the abdominal cavity

(ascites) and legs due to liver damage. Kidney disease results in the retention of extra fluid and sodium, primarily causing edema in the legs and around the eyes[3].

Edema can also arise from a compromised lymphatic system, responsible for removing excess fluid from tissues. Damage, such as from cancer surgery affecting lymph nodes and vessels, can disrupt fluid drainage and cause edema. Additionally, prolonged severe protein deficiency in the diet can result in fluid build-up and edema over time. In summary, edema can manifest as a symptom of diverse conditions affecting various bodily systems, underscoring the importance of identifying and addressing the underlying cause[4].

Buccal administration is a topical drug delivery route in which medications placed or applied in the buccal area (inside the cheek) permeate through the oral mucosa, entering directly into the bloodstream[5]. Buccal delivery involves drug discharge when a dosage form is positioned between the buccal mucosa and gingiva in the outer vestibule. Buccal tablets adhere and soften on the buccal mucosa, remaining in place until dissolution and discharge are complete[6]. This study aimed to formulate and evaluate mucoadhesive buccal tablets of Torsemide (MBT) using mucoadhesive polymers like HPMC K100, Xanthan Gum, and chitosan via direct compression. The objective was to enhance Torsemide bioavailability, bypass hepatic first-pass metabolism, ensure a predictable and extended duration of action, reduce undesirable effects, extend Torsemide residence time in the body, and promote patient compliance due to painless Torsemide delivery. This research seeks to optimize the Torsemide delivery method for improved therapeutic outcomes.

2. Materials And Methods

Material

Torsemide was acquired as a gift sample from Micro Labs Limited in Bangalore. HPMC K-100, Xanthan Gum, and Chitosan were sourced from S.D. Fine Chemicals Pvt. Ltd. in Mumbai, India. All other chemicals and solvents utilized in the study were of standardized analytical grades.

Pre formulation Studies

Solubility

Assessing the solubility of the selected drug involved a systematic examination across a range of solvents. This encompassed distilled water, methanol, 0.1N HCl, 0.1N NaOH, and a phosphate buffer at pH 6.8. The methodology employed adhered to established standards for such evaluations[7].

Melting Point

A glass capillary tube filled with the Torsemide, sealed at one end, was attached to a thermometer using a thin thread. This assembly was placed within a Thiele tube, and the Thiele tube was immersed in liquid paraffin. The capillary tube was positioned so that its lower tip just touched the liquid paraffin. The heat was applied, and the temperature at which the Torsemide began to melt was recorded. This process allowed the determination of the melting point of the Torsemide[8].

Estimation of Torsemide for absorption maxima

The preparation of the Torsemide stock solutions involved a sequential procedure. Initially, Torsemide stock solution I was created by dissolving 100 mg of Torsemide in 100 ml of phosphate buffer with a pH of 6.8, resulting in a concentration of 1 µg/ml. Subsequently, a serial dilution was carried out using phosphate buffer pH 6.8 within a 100 ml volumetric flask, ultimately yielding stock solution II with a concentration of 100 µg/ml. From stock solution II, an aliquot was withdrawn and further diluted with phosphate buffer pH 6.8 to achieve a concentration of 5 µg/ml. This diluted solution was then subjected to UV spectrophotometric analysis within the wavelength range of 200-400 nm, utilizing a UV spectrophotometer. During this analysis, the diluted solution was compared against a blank to determine its optical properties and characteristics[9]. This methodology was applied to derive valuable insights into the absorbance and spectral behavior of Torsemide within the specified wavelength range.

Determination of Calibration Curve of Torsemide

A stock solution, designated as Stock Solution I, was meticulously prepared to contain Torsemide at a concentration of 1 mg/ml. To further the analysis, this primary stock solution underwent serial

dilution, culminating in the formation of Stock Solution II, with a Torsemide concentration of 100 µg/ml. From this newly generated Stock Solution II, precise volumes of 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml were meticulously pipetted out and subsequently transferred into distinct 10 ml volumetric flasks. In a carefully orchestrated sequence, these volumes were subsequently subjected to dilution utilizing phosphate buffer with a pH of 6.8. This systematic process resulted in the creation of a set of solutions, each bearing concentrations of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, and 10 µg/ml, respectively. The subsequent phase of the experiment involved the measurement of the absorbance of each of these derived solutions[10]. This measurement was carried out at a specific wavelength of 284.5 nm, using a specialized instrument known as a spectrophotometer. Notably, each solution's absorbance was gauged against a blank, facilitating accurate determination[11]. This meticulous series of dilutions and subsequent absorbance measurements served a dual purpose: first, they contributed to the establishment of a precise calibration curve, a vital tool for correlating concentration with absorbance values; secondly, they offered a means to gauge and quantify the concentration of Torsemide within the solutions based on their unique absorbance responses at the specified wavelength.

Compatibility Studies using FTIR

An FTIR analysis was conducted to assess the compatibility between the Torsemide and excipients using the potassium bromide pellet method. To prepare the sample disc, approximately 1-2 mg of the sample substance was triturated with around 10-20 mg of KBr. This mixture was then compressed using a hydraulic press to form a thin disc with a diameter of roughly 10-15 mm. The resulting disc was subjected to scanning within the range of 4000 to 400 cm^{-1} using an FTIR spectrophotometer. The disc was placed in the sample holder during the scanning process. By comparing the obtained spectra of the Torsemide and the excipients, functional group peaks were analyzed and interpreted to identify any potential interactions. This FTIR analysis aimed to provide insights into the chemical compatibility and interactions between the components of the formulation.

Evaluation of MBT powder blend

The flow features and compressibility of the powder blend were assessed through several parameters using the fixed funnel method. The angle of repose, bulk density, tapped density, Hausner's ratio, and compressibility index were measured to understand the powder blend's behavior[10]. These measurements provide insights into the powder's flowability and compressibility properties, which are essential for formulation and processing considerations.

Preparation of MBT

The MBT were formulated through a direct compression method, employing HPMC K100, Xanthan Gum, and Chitosan as mucoadhesive polymers. In adherence to the batch formula, precise weights of the Torsemide, polymers, and all excipients were measured. The procedure involved sequentially blending all components except for the lubricant and glidant. This mixture was triturated in a glass mortar for 15 min. Subsequently, the lubricant and glidant were incorporated, followed by an additional 3 min mixing[12]. The resultant powder blend was then compressed into tablets using 8 mm round flat punches, with the MBTs formed based on their designated weights as per the formulation[13]. This method aimed to create mucoadhesive MBTs with consistent composition and properties for targeted buccal administration (Table 1).

Table 1: Formulation of MBT

Ingredients	Formulations							
	F-1	**F-2**	**F-3**	**F-4**	**F-5**	**F-6**	**F-7**	**F-8**
Torsemide	20	20	20	20	20	20	20	20
HPMC K100	30	35	40	45	-	-	-	-
Xanthan gum	-	-	-	-	5	10	15	20
Chitosan	10	10	10	10	10	10	10	10
Mannitol	80	85	70	65	105	100	95	90
Sodium CMC	10	10	10	10	10	10	10	10
Magnesium stearate	5	5	5	5	5	5	5	5

Talc	5	5	5	5	5	5	5	5
Ethylcellulose	40	40	40	40	40	40	40	40
Total	200	200	200	200	200	200	200	200

Evaluation of Buccal MBT

The MBT underwent a series of evaluations encompassing the following tests:

Weight Variation Test

A random selection of 20 MBT was chosen and individually weighed, enabling the calculation of average tablet weight. By comparing the individual weights to the calculated average weight, the extent of weight variance among the MBT assessed[14].

Hardness

The ability of tablets to withstand shipping, handling, and storage conditions without breaking is dependent on their strength and hardness. To assess this, the Pfizer hardness tester was employed. Ten randomly chosen buccal tablets were subjected to measurement using this device, which quantifies the pressure needed to fracture the tablets by applying force with a coiled spring. The results are expressed in Kg/cm², providing insights into the tablets' mechanical strength and their capacity to endure various stresses[14].

Friability

The assessment of tablet friability was conducted using the Roche Friabilator apparatus, commonly referred to as a Friabilator. In this evaluation, a sample of 10 tablets was randomly selected from each batch of Mucoadhesive Buccal Tablets (MBTs). These tablets underwent a standardized process wherein they were subjected to rotational motion at a speed of 25 revolutions per minute (rpm) for a duration of 4 minutes. During this rotational process, each Mucoadhesive Buccal Tablet was repeatedly dropped from a consistent height of 6 inches with each full revolution. Over the course of 100 complete rotations, the tablets experienced this cyclic impact and abrasion. Following the completion of these 100 revolutions, the tablets were carefully dedusted to remove any particulate matter adhering to their surfaces. Subsequently, the tablets were reweighed, and the extent of weight loss was calculated as a percentage. This calculation involved comparing the post-test weight to the initial weight of the tablets. This percentage of weight loss is a key indicator of friability and provides insights into the tablets' resistance to both abrasion and impact. This test serves as a valuable assessment of the MBT's structural integrity and their susceptibility to fragmentation during various stages of handling, transportation, and eventual use[15]. The results of this friability test aid in determining the tablets' potential for withstanding stressors that might be encountered in real-world scenarios, contributing to the tablets' overall quality evaluation and assurance (e.q.1).

$$\% \text{ Friability} = \frac{Winitial - W final}{Winitial} X100 \quad \text{--- (1)}$$

Thickness and Diameter

The dimensions of the formulated MBTs were assessed using a digital Vernier caliper. In this process, five MBTs were selected from each representative MBT. The thickness and diameter of each MBT were measured individually. The average of these measurements was then calculated to determine the mean MBT thickness and mean MBT diameter for each batch. This measurement procedure provides information about the MBT's physical dimensions, which are critical for consistent dosing and uniformity in pharmaceutical preparations[16].

Torsemide Content

In this specific protocol, a total of 10 MBTs from each batch underwent a preparation process for analysis. The tablets were meticulously ground using a mortar and pestle, resulting in a powdered form. From this powdered material, an amount equivalent to the mass of a single MBT was extracted and combined with a suitable quantity of phosphate buffer at pH 6.8. The ensuing mixture was then subjected to filtration through a Whatman filter paper, a process employed to eliminate any solid

particles or insoluble components. This filtration step facilitated the separation of the liquid phase, known as the filtrate, from the solid residues. The obtained filtrate was then subjected to further dilution with additional phosphate buffer at pH 6.8. This diluted filtrate, now suitably prepared for analysis, underwent assessment for Torsemide content. This evaluation was conducted by measuring the absorbance of the solution at a specific wavelength of λmax 284.5 nm using a specialized instrument called a UV spectrophotometer[17,18]. To enable accurate quantification, a calibration curve was generated using a standard reference compound, Zolmitriptan. This curve, derived from known concentrations of Zolmitriptan, facilitated the correlation between absorbance and concentration[19]. The application of this calibration curve allowed for the determination of Torsemide content in the formulated Mucoadhesive Buccal Tablets. The accurate assessment of Torsemide concentration in these tablets holds significant importance, serving as a crucial aspect of quality control and ensuring proper dosing. This meticulous procedure guarantees the reliability and precision of Torsemide content measurements in the formulated tablets, ultimately contributing to the effectiveness and safety of the pharmaceutical product[20].

Surface pH

To ascertain the potential impact on the buccal mucosa, the surface pH of the developed MBTs was meticulously assessed. This step aimed to evaluate the likelihood of any discomfort that might arise upon contact with the buccal mucosa. The significance of this assessment lies in maintaining a surface pH that is either neutral or aligned with the range of salivary pH, a pivotal factor in preventing irritation to the buccal mucosa. In order to execute this evaluation, a specialized combined glass electrode was employed[21]. The MBTs under consideration were brought into contact with 1 ml of phosphate buffer, characterized by a pH value of 6.8[22]. This interaction was allowed to transpire over a duration of 2 h, a timeframe that facilitated the swelling of the MBTs. Upon reaching equilibrium, the combined glass electrode was strategically positioned to make contact with the expanded Mucoadhesive Buccal Tablet. Following a brief period of equilibration, precisely one minute after this equilibrium was achieved, the pH was measured. This measurement serves as a pivotal indicator of the MBTs' surface pH and their potential compatibility with the buccal environment. By undergoing this assessment, the MBTs' ability to maintain a pH level conducive to patient comfort during administration is gauged. The careful attention to surface pH underscores the dedication to producing pharmaceutical products that prioritize patient experience and minimize the potential for irritation to the sensitive buccal mucosa.

Swelling Index Study

The swelling index (SI) of the buccal MBTs was assessed in phosphate buffer at pH 6.8 to determine their ability to absorb moisture and expand, crucial for buccal administration and Torsemide release kinetics[23]. The process involved measuring the initial weight (W_1) of each MBT, placing them in 5 ml of pH 6.8 phosphate buffer, and incubating at 37°C. At specific time intervals (1, 2, 3, 5, 6, 7, and 8 h), the MBTs were removed, gently blotted to remove surface moisture, and reweighed (W_2)[24]. The SI, indicating the extent of swelling, was calculated by comparing W_1 and W_2. This study offers insights into the MBTs' moisture absorption and expansion behavior, pertinent for their effective use in buccal administration and controlled Torsemide release (e.q.2).

$$\% SI = \frac{W2-W1}{W1} X100 \quad \text{--- (2)}$$

***In-vitro* Torsemide Discharge Studies**

The in-vitro release of Torsemide from the MBTs was investigated using the USP type-II rotating paddle method. The experiment was structured as follows: A total of 900 ml of phosphate buffer with a pH of 6.8 served as the dissolution medium. The dissolution vessel incorporated a bottom disc. The study was executed at a consistent temperature of 37±0.5°C, and a rotational speed of 50 revolutions per minute (rpm) was maintained. To ensure appropriate conditions, regular samples of the dissolution medium were drawn at specified intervals, and fresh buffer solution was introduced to maintain sink conditions[25]. Collected samples were subjected to filtration using Whatman filter paper. From each

filtered sample, a volume of 1 ml of the filtrate was extracted and subsequently diluted to a total volume of 10 ml, using phosphate buffer at pH 6.8[26]. These diluted samples underwent analysis for absorbance at a specific wavelength of 284.5 nm. The percentage of cumulative Torsemide release was calculated through reference to a Torsemide calibration curve. This curve allowed for a correlation between absorbance values and known Torsemide concentrations. The in-vitro release study offered profound insights into the Torsemide discharge profile from the Mucoadhesive Buccal Tablets under the specific dissolution conditions. This endeavor contributed to the comprehension of the MBT's performance and the kinetics of its drug discharge. Ultimately, this investigation assists in characterizing the behavior of the MBTs in relation to drug release, thereby aiding in the MBT's optimization and enhancing the understanding of its therapeutic potential.

Mucoadhesive Strength

The evaluation of mucoadhesive strength involved a method that measured the force required to detach a MBT from sheep buccal mucosal tissue. Fresh buccal mucosa was sourced from a local slaughterhouse within a 3h timeframe post-slaughter. The mucosa was meticulously cleansed with distilled water and subsequently rinsed using phosphate buffer at a pH of 6.8. The assessment procedure incorporated the utilization of a double-beam physical balance, which featured a suspended thread connected to a glass stopper on its left arm. The buccal mucosal membrane was securely positioned, with the mucosal side facing upwards, on the inverted base of a 50 ml glass beaker. This setup was contained within a larger 500 ml beaker filled with phosphate buffer at a pH of 6.8, maintained at a temperature of 37±2°C to preserve mucosal moisture. To initiate the procedure, the balance was initially equalized. Weights on the right-hand pan were meticulously adjusted to create a 5 g reduction[27]. This reduced weight was then gradually decreased to lower both the MBT and the attached glass stopper onto the mucosal surface. This configuration was sustained for a period of three minutes (as depicted in Figure 1). Subsequently, weights were progressively reintroduced to the right pan until the point where the MBT eventually separated from the mucosal membrane[28]. The mucoadhesive strength was quantified by determining the additional weight required on the right pan, which amounted to the total weight minus the initial 5 g reduction. Throughout the assessment process, multiple trials were conducted, and between each measurement, the buccal tissue was thoroughly rinsed. This comprehensive approach was instrumental in evaluating the MBTs' capability to firmly adhere to the mucosal surface[29]. Such adhesion is of paramount importance for the effective discharge and administration of Torsemide, thus underlining the crucial role of mucoadhesive strength in pharmaceutical development (e.q.3).

$$\text{Force of Adhesion (N)} = \frac{\text{Mucoadhesive Strength}}{1000} X9.8 \quad \text{--- (3)}$$

Fig.1. Modified balance for mucoadhesion

***In vitro* Torsemide discharge kinetics**

The *in-vitro* discharge data were subjected to various kinetic models to analyze the Torsemide discharge kinetics. These models included the Zero-order model, where % Torsemide released was plotted against time; the First-order model, where log % cumulative Torsemide released was plotted against time; the Higuchi model, where % cumulative Torsemide discharged was plotted against time; and the Korsmeyer-Peppas model, where log % Torsemide discharged was plotted against log time. By calculating r^2-values, which reflect the goodness of fit, the most suitable model was determined. The model with the highest r^2-value was selected as the best-fit model, providing insights into the Torsemide discharge mechanism and kinetics from the MBTs[30].

3. Results and Discussion

Solubility

The investigation into Torsemide's solubility encompassed a range of solvents, shedding light on its dissolving behavior. Notably, Torsemide displayed solubility in both methanol and phosphate buffer with a pH of 6.8. Furthermore, it exhibited the characteristic of being highly soluble in 0.1N NaOH solution, while demonstrating slightly soluble characteristics in water. This comprehensive assessment provides valuable insights into Torsemide's solubility profile, aiding in understanding its dissolution behavior in various contexts.

Melting Point

The melting point of Torsemide was ascertained using the Theile tube method, yielding a value of 159±2.3°C, which aligns with the specified range in the Torsemide monograph.

Determination of λ_{max}

The prepared standard solution of Torsemide at a concentration of 10 µg/ml underwent spectral analysis within the wavelength range of 200 to 400 nm. The analysis revealed that the solution exhibited its highest absorbance peak at a specific wavelength of 284.5 nm, as depicted in Figure 2. This absorption pattern serves as a distinctive fingerprint, offering valuable information about Torsemide's response to light across this spectral range (Figure 3).

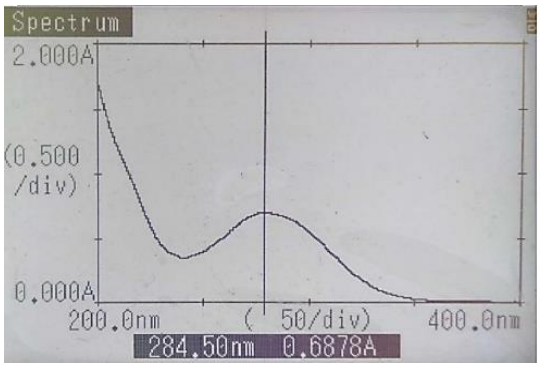

Fig.2: Absorbance maxima of Torsemide.

Standard calibration curve

A standard calibration curve for Torsemide was constructed in phosphate buffer at pH 6.8, resulting in a linear equation of y = 0.0208x + 0.013 with a regression coefficient (r^2) value of 0.9981 (Figure 3A). This linearity exhibited adherence to the Beer-Lambert rule, indicating the accurate relationship between concentration and absorbance.

Torsemide-Excipient Compatibility Study

The compatibility study was carried out by FTIR spectroscopy and studied for interaction through the following FTIR spectra peaks. By using FTIR Spectroscopy, Torsemide-polymer compatibility

studies were conducted to determine any potential interactions between excipients and the Torsemidein the MBTs. For the compatibility study, the FTIR spectrum of the Torsemide alone and in combination with polymers was determined. The primary absorption peaks for the pure drug were found to be at 3336.42 cm^{-1} (N-H), 2944.23 cm^{-1} (C-H), 3411.55 cm^{-1} (N-H), 1139.87 cm^{-1} (SO_2), and 990.34 cm^{-1} (C-N), respectively. The spectra of the physical mixture, which contains the Torsemide and excipients, showed vibrations as well. Since it displays the distinctive peak of Torsemide and excipients, the FTIR study demonstrated that there are no significant Torsemide-excipients interactions (Figure 3).

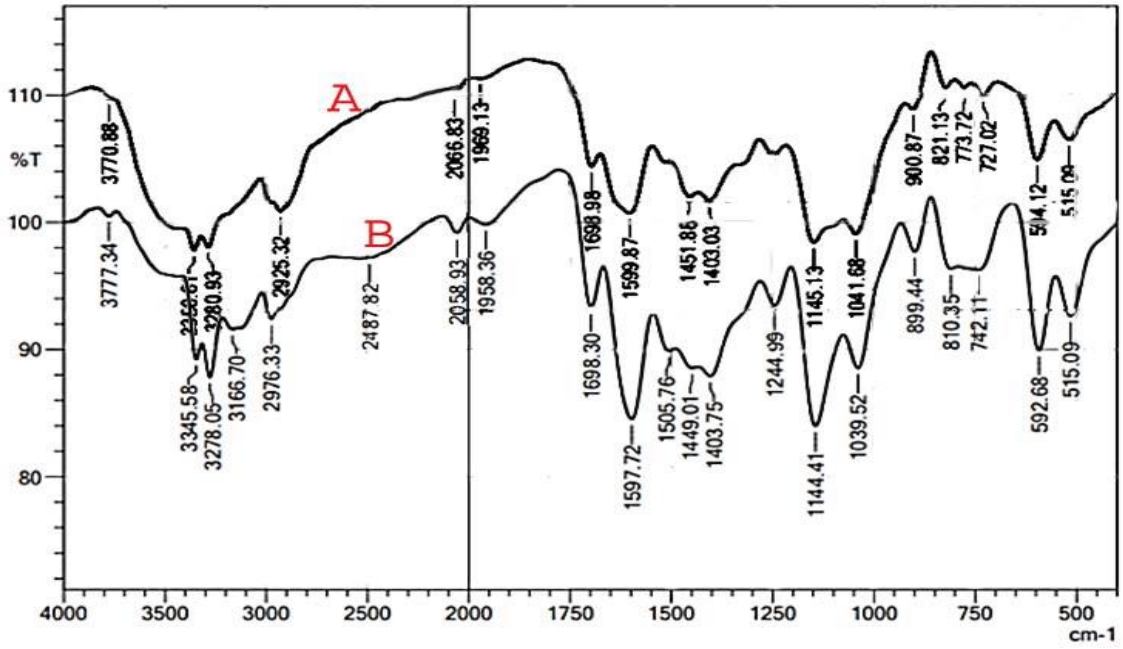

Fig.3. FTIR Spectrum of A) Torsemide B) Torsemide with excipients

Pre- compression evaluation parameters

The assessment of the MBT's properties revealed promising results. The angle of repose ranged from 25.97º±0.65 to 31.08º±1.57, indicating favorable flow features due to the relatively low angles observed. Furthermore, Carr's index values spanning from 6.27±2.98 to 18.89±1.21 suggested reasonable flowability, albeit with a slight variation. Hausner's ratio, ranging between 1.06±0.03 and 1.23±0.08 (Table 2), reinforced the MBT's compressibility and satisfactory flow attributes. Collectively, these measurements underscored the MBT's potential for processes requiring excellent flow behavior and compressibility.

Table 2: Pre-compression evaluation parameters of the powder blend

Formulation	Angle of repose (^{O})	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Hausner's ratio
F1	26.97 ±1.92	0.304±0.03	0.35±0.02	12.54±2.46	1.14±0.02
F2	27.04±1.72	0.299±0.02	0.32±0.02	6.27±2.98	1.06±0.03
F3	27.65±0.84	0.304±0.03	0.35±0.01	13.74±1.89	1.15±0.05
F4	31.08 ±1.57	0.305±0.02	0.38±0.05	18.89±1.21	1.23±0.08
F5	29.94 ±1.25	0.300±0.03	0.36±0.03	15.89±1.45	1.18±0.02
F6	28.10±1.28	0.304±0.02	0.35±0.02	13.51±0.57	1.15±0.07
F7	25.97 ±0.65	0.315±0.02	0.39±0.01	18.27±2.66	1.22±0.04
F8	30.05±2.29	0.305±0.01	0.35±0.02	11.54±2.18	1.13±0.08

Values in mean±SD

Post-compression evaluation parameters

The MBT thickness remained consistent, spanning from 3.8±0.27 to 4.1±0.15 mm. This uniformity suggested a reliable manufacturing process. In terms of weight variation, all MBTs successfully passed the test, with the average percentage deviation falling within the acceptable range of ±7.5% as specified by pharmacopoeia standards. The tMBTs exhibited a range of friability values from 0.245±0.207 to 0.941±0.006, all comfortably within the approved threshold of 1%. This affirmed their ability to withstand mechanical stress and abrasion. Notably, the MBTs displayed hardness levels ranging from 5.32±0.19 to 5.72±0.39 kg/cm², aligning with the standard value of > 4 kg/cm² standard. This indicated commendable mechanical strength. The MBTs' surface pH values, ranging from 6.7±0.05 to 6.9±0.11, fell within the range of the buccal mucosa's pH, ensuring avoidance of local mucosal irritation. Moreover, the percentage Torsemide content spanned from 84.78±4.69% to 99±0.19% (Table 3), well within the established pharmacopoeia standards. This comprehensive assessment underscores the MBTs' robust physical and chemical attributes, positioning them favorably for their intended use.

Table 3: Post-formulation parameters of MBTs

Formulation	Thickness (mm)	Weight Variation (mg)	Friability (%)	Hardness (Kg/cm^2)	Torsemide Content (%)	Surface pH
F1	3.9±0.22	198±1.15	0.475±0.02	5.62±0.16	90.86±4.34	6.8±0.05
F2	4.0±0.15	196±1.08	0.941±0.06	5.68±0.31	99.00±0.19	6.7±0.05
F3	3.8±0.27	197±1.15	0.640±0.37	5.62±0.13	95.35±4.09	6.9±0.05
F4	3.9±0.28	196±0.57	0.275±0.19	5.60±0.22	91.74±6.20	6.8±0.05
F5	4.0±0.01	197±1.52	0.245±0.20	5.72±0.39	86.62±2.87	6.8±0.05
F6	3.9±0.22	198±1.52	0.764±0.25	5.52±0.22	91.27±1.56	6.9±0.20
F7	3.8±0.27	197±1.15	0.486±0.32	5.64±0.28	84.78±4.69	6.9±0.11
F8	4.1±0.15	198±1.12	0.720±0.20	5.32±0.19	93.59±0.84	6.9±0.10

Values in mean±SD

Swelling Index

The swelling index (SI) study was carried out by using phosphate buffer pH 6.8. The SI of all MBTs were evaluated by calculating the SI at various time intervals. Which indicates swelling increases with time. Among all formulations, F6 containing Chitosan and Xanthan gum as polymer shows the highest SI of 241.9 ±15.5 % and F4 containing Chitosan and HPMC-K100 shows the lowest SI of 201.8±5.9% (Figure 3B).

Kinetic modelling of the Torsemide discharge

To decipher the pattern of Torsemide discharge, the in-vitro release data were subjected to fitting with various kinetic models, encompassing zero order, first order, Higuchi, and Korsemeyer-Peppas equations. Among the MBTs studied, namely F2, F3, F4, F5, F6, F7, and F8, they all conformed to zero order kinetics with r^2 values of 0.9237, 0.9866, 0.9419, 0.9452, 0.9667, 0.9388, and 0.9818. This observation indicates that the rate of discharge in these MBTs remains independent of concentration. For MBT F1, the r^2 value was determined to be 0.9566. This points toward a discharge pattern regulated by both diffusion and erosion processes. Furthermore, as the diffusion exponent of the solute (n) exceeded 0.89, it is evident that the mechanism underlying Torsemide discharge is characterized as super case II transport. This intricate analysis of kinetic models not only sheds light on the discharge patterns of various MBTs but also unveils critical insights into the underlying mechanisms governing Torsemide's release from the MBTs (Figure 4C, 4D, 4E, and 4F).

Table 4: Kinetic modelling plot of MBTs (F1-F8)

Formulation	Zero-order	First order	Higuchi	Korsmeyer- Peppas	
				r^2	n
F1	0.9566	0.9575	0.9862	0.985	0.5638
F2	0.9237	0.8936	0.9934	0.9933	0.563

F3	0.9866	0.9524	0.984	0.9914	0.7144
F4	0.9419	0.8860	0.9817	0.9820	0.6475
F5	0.9452	0.7974	0.9603	0.9366	0.7345
F6	0.9667	0.9617	0.9355	0.9614	0.6179
F7	0.9388	0.8742	0.9598	0.9671	0.7026
F8	0.9818	0.9601	0.9485	0.9557	0.7584

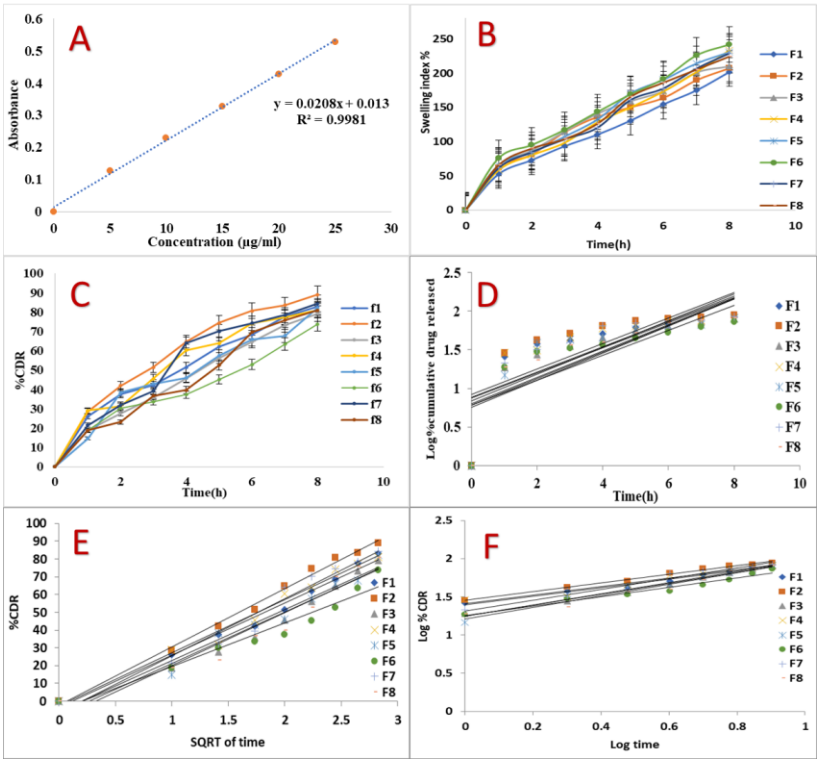

Fig.4: A) Standard calibration curve of Torsemide; B) Swelling index; C) Zero-order discharge plots; D) First-order discharge plots; E) Higuchi plots; F) Korsmeyer Peppas plots for the formulations F1-F8

Ex-vivo mucoadhesive strength

The degree of SI of the polymers, contact time with buccal membrane, and molecular weight of the polymer all have an impact on the mucoadhesive strength. The MBT employing chitosan and HPMC-K100 as the polymer produced the highest mucoadhesive strength of 27.33±2.08g. Due to these polymers' noticeable swelling after being hydrated, they exhibited the highest mucoadhesive strength. Due to its minimal swelling propensity and quick detachment after hydration, Chitosan and Xanthan Gum MBTs had the lowest mucoadhesive strength 18.00±1g (Figure 5A and 5B).

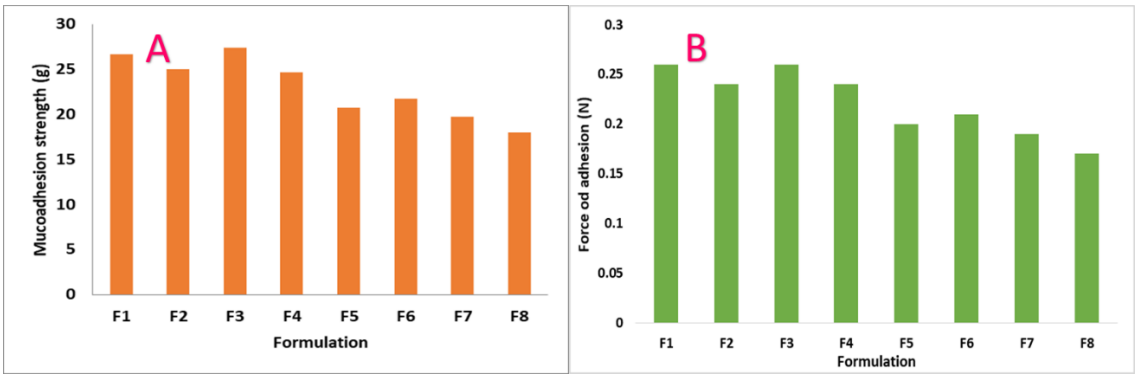

Fig.5. A) Mucoadhesive strength; B) Force of adhesion of MBTs (F1- F8).

The solubility analysis of Torsemide revealed its high solubility in phosphate buffer at pH 6.8 and methanol, along with being freely soluble in 0.1N NaOH and slightly soluble in distilled water. This comprehensive analysis played a pivotal role in selecting appropriate solvent systems for Torsemide dissolution and identifying the optimal dissolution medium. The determined average melting point of Torsemide at 159°C fell within the range specified by the Indian Pharmacopoeia (I.P.), underscoring its purity and absence of impurities. The successful construction of a calibration curve for Torsemide estimation using phosphate buffer at pH 6.8 and a wavelength of 284.5 nm was a significant achievement. This method adhered to Beer-Lambert's law within the range of 2 – 10 µg/ml, displaying a robust r2 value (>0.99) and minimal standard deviation. These results collectively indicated high reproducibility and suitability for accurate Torsemide estimation. Compatibility studies conducted through FTIR revealed that the characteristic peaks of Torsemide remained unaltered when combined with polymers HPMC K100, Chitosan, and Xanthan Gum. This outcome indicated that Torsemide remained in its pure form, without significant interactions occurring between the drug and the polymers during the formulation process. Key parameters in powder property evaluation, including bulk density, tapped density, Carr's index, Hausner's ratio, and angle of repose, collectively indicated favourable flow properties and efficient compressibility. These attributes are crucial for consistent and smooth manufacturing processes, particularly in industries reliant on consistent powder behaviour.

Physical measurements such as thickness and diameter aligned well within acceptable parameters for the tablet compression machine die size. Additionally, MBT hardness exceeding 4 kg/cm² and friability loss remaining below 1% highlighted robust mechanical strength across MBTs. Uniform weight distribution was confirmed across different MBT batches, adhering to Indian Pharmacopoeia standards. Torsemide content uniformity was also verified, ensuring consistent dosing within each MBT. Furthermore, the MBTs displayed a near-neutral surface pH, falling within the suitable range for buccal administration while minimizing the potential for mucosal irritation.

The swelling index investigation conducted in phosphate buffer at pH 6.8 demonstrated significant swelling characteristics of the prepared buccal MBTs over an 8h span. In the *in-vitro* Torsemide release study, MBT- F6, featuring Chitosan and Xanthan Gum, showcased the most substantial Torsemide release within 8 h compared to otherMBT. The mucoadhesive strength was most pronounced in the MBT containing Chitosan and HPMC K100, attributed to its notable swelling behavior. Conversely, the MBT containing Chitosan and Xanthan Gum showed lower mucoadhesive strength due to reduced swelling and quicker detachment upon hydration.

4. Conclusion

This study developed Torsemide mucoadhesive buccal tablets using HPMC K100, Chitosan, and Xanthan gum, individually and in combinations, for Edema treatment. FTIR tests confirmed no Torsemide-excipient interactions. Physicochemical parameters met pharmacopoeia standards. Formulations showed favorable swelling and non-irritating pH values. Notably, Formulation F3 (HPMC K100 and Chitosan) exhibited strong mucoadhesion, sustained Torsemide release, and aligned with zero-order and Korsemeyer Peppas models, showcasing potential for effective Edema therapy.

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