JOURNAL OF ADVANCED MEDICAL AND PHARMACEUTICAL RESEARCH

Research Article

In-Vitro Characterization and In-Vivo Pharmacokinetic Study of Zolmitriptan Mucoadhesive Buccal Film for Expedited Migraine Relief

Received 11th September 2024,
Accepted 1st December 2024
Published 18th December 2024

10.21608/jampr.2024.320092.1078
jampr.journals.ekb.eg

Omar Mady[1], Dalia H. Abdelkader[1], Sara Hussien[1], Enas El Zahaby[2]*

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Tanta University, Tanta, Egypt.
[2]Department of Pharmaceutics, Faculty of Pharmacy, Delta University for Science & Technology, Egypt.

ABSTRACT

The study aims to address the need for an efficient and patient-friendly alternative for migraine relief. Utilizing Zolmitriptan (ZMT) -loaded buccal films offers a promising avenue for rapid onset of action and enhanced efficacy compared to traditional tablets. Film formulations were prepared and analyzed for ZMT content, mechanical properties, pH values, thickness, amorphization through Differential Scanning Calorimetry, and moisture uptake at varying drug loading. The swelling index and adhesion properties were also investigated. In vitro dissolution profiles were determined. In vivo studies assessed Tmax enhancement compared to traditional tablets. ZMT buccal films demonstrated ZMT content between 2.38±0.19 and 2.46±0.16 mg/film, with optimal mechanical properties and pH values suitable for oral cavity application. DSC analysis highlighted ZMT amorphization during preparation, and moisture uptake decreased with higher drug loading. Concentration-dependent effects were observed in the swelling index and adhesion properties. In vitro studies revealed rapid erosion and quicker drug release. In vivo studies demonstrated a significant shortening in Tmax (from 1.33 ± 0.61 to 0.63 ± 0.26 hr) compared to traditional tablets. The prepared ZMT-loaded mucoadhesive buccal film showcases immense potential as a patient-friendly alternative for swift alleviation from migraine episodes. The findings underscore the formulation's robustness, demonstrating enhanced drug release and significant clinical advantages over conventional treatments.

Keywords: Mucoadhesive; Zolmitriptan; Pharmacokinetics; Bioavailability; Carboxymethyl cellulose (CMC); Buccal delivery.

1. INTRODUCTION

Migraine, a neurological condition, surpasses the combined prevalence of several CNS diseases such as epilepsy, Parkinsonism, and Alzheimer's (1). It manifests as intense headache episodes, characterized by either bilateral or unilateral discomfort ranging from mild to severe pain. Nausea, photophobia, and phonophobia (sensitivity to light and sound) are common symptoms accompanying migraine attacks. The effectiveness of migraine management could be assessed by decreasing the degree of headache pain from severe/ moderate to mild/negligible levels within a maximum of two hours post-drug administration. Therefore, medications employed for alleviating migraine-associated pain should demonstrate a rapid initiation of action. Triptans are a group of drugs used to treat migraines and cluster

**Department of Pharmaceutics, Faculty of Pharmacy, Delta University for Science & Technology, Egypt.*
E-mail address: enas.elzahabi@deltauniv.edu.eg

headaches by constricting blood vessels and reducing inflammation in the brain. Sumatriptan (Imitrex) was first approved by the FDA in 1992 and is available in various formulations such as oral tablets, injections, nasal sprays, and skin patches (2,3). Other triptans include naratriptn, rizatriptan, eletriptan, frovatriptan, and zolmitriptan. Zolmitriptan (ZMT), is a 5-HT1B and 5-HT1D receptor agonist considered for controlling acute migraine episodes with or without aura, particularly in women suffering monthly migraine combined with their mensural cycle (4). The pharmacological mechanism of ZMT is to inhibit the exaggerated dilatation of the arteries (intracranial and extracerebral), which relieves headache pain and related symptoms. ZMT is classified as a Class II drug showing poor aqueous solubility (5). The medication is commercially available in a 2.5 mg dosage as conventional tablets, prescribed for administration. The oral dosage form exhibits an approximate 40% mean absolute bioavailability for the parent compound, primarily attributed to its poor aqueous solubility. In addition to the drug's limited bioavailability, another significant drawback of the Zolmitriptan tablet is the delayed relief of migraine pain, which typically occurs three hours after oral administration (6).

Commercially available ZMT formulations include tablets, orally disintegrating tablets, and nasal sprays (2). Regarding the nasal route, it may cause irritation and discomfort in some patients. The buccal route of administration, on the other hand, offers a promising alternative for quick onset of action and bypassing intestinal and/or hepatic first-pass metabolism. Concerning the buccal delivery route, it provides numerous exceptional advantages such as a substantial smooth muscle expanse and comparatively static mucosa, making it appropriate for modified-release dosage forms with a higher rate of patient acceptability (7).

Thus, the primary objective of this inquiry is to assess the feasibility of utilizing the buccal route for the efficient delivery of ZMT, by formulating mucoadhesive buccal films composed of CMC and PG (aiming to enhance ZMT solubility) with varying drug loading. The films underwent a comprehensive *in vitro* analysis, encompassing evaluation of physical characteristics, assessment of drug content, moisture absorption, swelling index, adhesive strength, and *in vitro* dissolution profiles. Following this, a human volunteer-based *in vivo* study was executed to compare the pharmacokinetic parameters of the optimized ZMT-loaded buccal film against a commercially available traditional tablet. The overarching goal is to achieve a rapid antimigraine response with ZMT via buccal administration, capitalizing on numerous advantages over conventional oral dosage forms.

2. METHODS

2.1. Materials

Zolmitriptan (ZMT) was acquired as a complimentary gift from Amoun Pharmaceutical Company, El Obour, Egypt. Propylene glycol (PG) was provided by BDH Chemical Ltd. in Poole, UK. Carboxymethyl cellulose (CMC) was purchased from El-Gomhouria Company, Tanta, Egypt. The solvents employed included methanol and acetonitrile (HPLC grade, sourced from Fisher Chemical UK), along with deionized water (Stakpure, Waters, USA). Blank plasma utilized in the study was obtained from volunteers in good health. All additional chemicals were of standard grade and utilized in their original form.

2.2. Preparation of the mucoadhesive Buccal Films

Various formulations of mucoadhesive buccal films shown in Table 1 were developed through the solvent-casting technique (8). The process involved dissolving a calculated accurate weight of CMC (9) in 40 ml of boiling water, followed by the addition of an appropriate quantity of propylene glycol (PG) (10). The polymeric solution was kept stirred and heated using a magnetic stirrer (VELP Scientifica, Usmate Velate, Italy, Europe) till the complete miscibility of PG. The polymer solution underwent cooling under constant agitation until it attained a temperature of 60°C. A predetermined amount of zolmitriptan (ZMT) was subsequently introduced into the previously cooled polymeric solution. The resultant mixture was poured into a petri dish with a diameter measuring 95 mm and subjected to drying at 55°C for 24 hours. The desiccated film was then sectioned into uniformly sized segments, each theoretically containing 2.5 mg of ZMT, 11.4 mg/cm² of CMC, and 0.03 mg/cm² of PG for the plain formulation.

Table 1. The composition of various mucoadhesive buccal films containing ZMT.

Formula	ZMT (mg/cm²)	CMC (mg/cm²)	PG (mg/cm²)
Plain	----	11.4	0.03
F1	0.625		
F2	0.833		
F3	1.25		
F4	2.5		

2.3. *In vitro* examination of the mucoadhesive buccal film

2.3.1. Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was employed to thermally analyze pure Zolmitriptan, the plain film, and the medicated films corresponding to F1 and F3 formulations. Each sample, accurately measured, was encapsulated in an aluminum container. The temperature

elevation commenced at 25°C and progressed to a maximum of 250°C, with a heating rate set at 10 ºC /min. (11).

2.3.2. Assessment of the Physical Characteristics of Buccal Films with Mucoadhesive Properties

2.3.2.1. Visual Inspection

All the prepared zolmitriptan mucoadhesive buccal films underwent visual assessments to evaluate their transparency, homogeneity, surface texture, and color. These properties were qualitatively analyzed by visual inspection.

2.3.2.2. Microenvironment pH

pH of all formulations should be kept near neutral values as possible. A microprocessor pH meter equipped with a combined glass electrode (Hanna Instruments, pH 211, Smithfield, RI, USA) was utilized. Different sections of each formulation (1×1 cm²) using a small amount of D.W (1 ml) have been damped. pH electrode has been kept in contact with the wetted sections for 2-4 minutes (12). The entire procedure was conducted in triplicate, and the standard deviation was calculated based on the average.

2.3.2.3. Weight and thickness uniformity

The square-shaped sections of each formula have been accurately cut to the same dimension (n=6), and then their weight uniformity has been determined gravimetrically (13) using a Digital balance (OHAUS electric balance; model PA413; USA). Furthermore, the thickness of the same square sections was measured using a Vernier Caliper (14) (15 mm × 0.05 mm, Poznań, Poland) to investigate the thickness uniformity. The mean results of both weight and thickness uniformity were expressed as the average value ± standard deviation.

2.3.2.4. Folding Endurance

The prepared films' folding endurance was evaluated by folding the films in the same position several times till the endpoint of the assessment. The endpoint is considered when the tested buccal film is broken or folded 50 times with no cracking. This procedure evaluates the durability and flexibility of the films (15).

2.3.3. Determination of content uniformity

A segment of the prepared films, comprising theoretically 2.5 mg of the drug (F1-F4), was excised, and dissolved in an appropriate volume of PBS (pH=6.8). Then, the solution was adequately diluted and analyzed using a UV/visible spectrophotometer (Thermo Fisher Scientific, model EVO 300PC, software: Vision Pro, Carlsbad, CA USA) at a wavelength of 223 nm. The measurements were conducted in triplicate, and the mean ±SD was documented. The effective drug content was subsequently computed regarding the theoretical drug content in the film segment (mg/film segment).

2.3.4. Moisture uptake

The moisture absorption capacity of the buccal films was assessed through a gravimetric approach (16). The weights of empty petri dishes used during film casting have been recorded. Their weights have been redetermined after buccal film preparation and drying at 55 °C for 24 hours to estimate the initial weights of the prepared buccal films. The dried films were left at room temperature and humidity, and their weights were redetected at different intervals up to 160 minutes. The percentage of weight gain in the films caused by the absorption of moisture was determined using the following Equation 1.

$$Moisture\ Uptake\ (\%) = \frac{Final\ Weight\ (t) - Inital\ Weight}{\text{Initial Weight}} x100 \quad (1)$$

2.3.5. Percentage swelling index

For assessing the swelling index of a film, a plastic mesh with numerous apertures of 50 µm diameter was employed. The mesh underwent an initial immersion in PBS (pH=6.8) for 5 minutes, followed by extraction and gentle shaking to eliminate any excess solvent. The precisely weighed film was placed onto the mesh, and the overall system weight was recorded initially as W_0. Subsequently, the whole system was reemerged into the same medium at predetermined time intervals for one hour. At each interval, the weight was documented as W_t. The swelling-erosion index was subsequently determined using Equation 2.

$$\text{Swelling Index} = \frac{wt - wo}{wo} x100 \quad (2)$$

Where wt and wo are the film's weight at time t and zero respectively.

2.3.6. In Vitro adhesion Strength

The mucosal membrane of a rabbit intestine as a tissue model was employed in this study to determine the adhesion strength. The intestinal membrane was gathered, cleaned, and subsequently preserved in PBS (pH=6.8). A two-arm balance apparatus was slightly adapted for this investigation (17). The rabbit gut was lightly thawed, then cut lengthwise, formed into square segments, and fixed onto a tissue holder with the use of cyanoacrylate adhesive. The surface of the mucosa was exposed, and a small amount of PBS was added. A pre-prepared buccal film, cut to fit the tissue holder's specifications, was positioned on the secured intestinal membrane of the slide. Both surfaces were allowed to make contact for a 2-minute preload duration, enhancing the adhesive effect. Afterward, the entire setup was hung

from a hook on the right balance arm, and a specific weight in a polyethylene bag was suspended from the left arm. Following the preload interval, water droplets were introduced into the polyethylene bag until separation occurred between the two surfaces. The gathered water mass was recorded and expressed as the force needed for detachment, referred to as adhesive strength. The equations used to determine the force of adhesion and bond strength (18) can be expressed differently as follows:

$$Force\ of\ adhesion\ (N) = \frac{[Bioadhesive\ strength\ (g) * 9.81]}{1000} \quad (3)$$

$$Bond\ strength\ (\mathrm{Nm^{-2}}) = \frac{[Force\ Of\ Adhesion\ ]}{Film\ Surface\ Area} \quad (4)$$

2.3.7. In Vitro ZMT dissolution study

To investigate the dissolution of ZMT from the developed buccal films, a paddle USP dissolution apparatus of Type Disc 6000 (manufactured by Copley Scientific, Colwick, UK) was employed. The experiment has been established in a controlled setting, utilizing 250 ml of PBS (pH=6.8), maintaining the operating temperature at 37 ± 0.5°C, and a rotational speed of 100 rpm. The buccal film, containing an equivalent of 2.5 mg of ZMT (as determined in section 2.3.3), was utilized for each run during the experiment. At predefined intervals, 5 ml of samples were removed and replaced with a similar volume of freshly prepared PBS of pH= 6.8 (19). Subsequently, the samples were analyzed using spectrophotometry at a wavelength of 223 nm. Each formulation was subjected to triplicate *in vitro* dissolution assessments.

2.4. Pharmacokinetic study (in humans):

An *in vivo* comparison has been performed between the optimized ZMT mucoadhesive buccal film (F4) and the reference conventional oral tablet (Amigrawest 2.5 mg, Western pharmaceutical industries with Batch No. BN22036). The loading of the two dosage forms was labeled to contain 2.5 mg of zolmitriptan. This study aims to investigate the variations of pharmacokinetic parameters following the administration of buccal film in comparison to conventional oral tablets in healthy volunteers.

2.4.1. Volunteers

For the comparative bioequivalence investigation, six male participants, in a good healthy state, were included in the study. Their length and weights were 175.6 ± 8.3 cm and 83.2 ±5.1 kg respectively. The well-being of the volunteers was examined through comprehensive medical evaluations, encompassing assessments of renal and hepatic function, a lack of any track record of drug misuse, recent blood donations, or the use of routine medications. Before and six hours after medication, competent nurses estimated the subjects' vital signs. Before beginning the trial, each volunteer completed and signed a written agreement form. During the study, no negative effects were disclosed.

Following international standards (as per FDA guidelines), the Faculty of Pharmacy, Tanta University approved the protocol of a two-way randomized, two-period, single-dose, single-blind, crossover bioequivalence investigation (TP/RE/11/22M-0067), incorporating a seven days interval in between the two stages. Prior to drug administration, participants were instructed to observe a 10-hour fasting period. The fasting period was extended for an additional two hours following the ingestion of the reference oral tablet and the application of the buccal film (test). After these two hours, participants were allowed to enjoy a typical breakfast consisting of light tea, white cheese, and toast. Post-drug administration, blood samples (3 ml each) were collected in heparinized glass tubes at intervals of 0, 0.25, 0.5, 0.75, 1, 1.5, 2.5, 4, 6, and 8 hours (as delineated in Table 2). The plasma, acquired through centrifugation (Harmonic series; DRE Universal, Kentucky, USA) at 3000 rpm for 10 minutes, was subsequently preserved (Binder, New York, USA) at -80 ºC till the samples' analysis.

During the study, volunteers received different treatments in each of the two periods. For instance, Volunteers 1 to 3 received Amigrawest oral tablet (Reference) during the first period and Buccal film during the second period. Similarly, Volunteers 4 to 6 received Buccal film (Test) during the first period and Amigrawest oral tablet during the second period.

2.4.2. Preparation of calibration curve

Primary stock solution (1000 µg/ml) of ZMT was prepared in methanol (20). Serially diluted concentrations were then prepared as working solutions ranging from 30 to 1000 ng/ml. Plasma samples (0.5 ml) spiked with 50 µl of the previously prepared working standard solution were employed to prepare 7 spiked plasma samples with the concentrations of 3, 5, 10, 20, 40, 50, and 100 ng/ml.

2.4.3. Samples analysis

The plasma samples (0.5 ml) collected from the volunteers at different time intervals (see section 2.4.2) were vortexed for approximately one minute with 1.5 ml of acetonitrile (HPLC grade), acetonitrile causing plasma protein to precipitate out (20,21).

Then, the samples were centrifuged (Harmonic series; DRE Universal, USA) at 3000 rpm for 15 minutes, after centrifugation, a protein pellet formed at the bottom of the tube. Subsequently, the resulting clear supernatants (1ml of supernatant) were transferred into a 10 ml volumetric flask and completed to volume with 0.1 N sodium dodecyl sulfate (22) and assessed using a modified version of a previously validated methodology, employing spectrofluorophotometry (Shimadzu RF-6000, Japan) with emission at 514 nm and excitation at 255 nm (23)

2.4.4. Pharmacokinetic and statistical analysis

Calculations of T_{max}, C_{max}, K_e, $T_{1/2}$, $AUC_{0\to t}$ and AUC_{total} were used to evaluate the study. According to the following equations, the trapezoidal formula was used to calculate $AUC_{0\to t}$. In the range of 3 to 100 ng/ml, all *in vivo* parameters were estimated for each volunteer and then reported as an average ± SD.

$$AUC_{0\to t}=\sum\left(\frac{C_n+C_{n+1}}{2}\right)\times(t_{n+1}-t_n) \quad (5)$$

$$AUC_{last}=\frac{C_{last}}{K_e} \quad (6)$$

$$AUC_{total}=AUC_{0\to t}+AUC_{last} \quad (7)$$

$$t_{1/2}=\frac{0.693}{k_e} \quad (8)$$

$$\text{Relative bioavailability}=\frac{AUC_{total\ test}}{AUC_{total\ reference}}\times 100\% \quad (9)$$

The impact of the period, treatment, sequences, and subject within the sequence was assessed using ANOVA. The results were statistically analyzed using the Minitab16 program on untransformed and logarithmically transformed data (C_{max}, $AUC_{0\to t}$ *and* AUC_{total}).

3. RESULTS AND DISCUSSION

The mucoadhesive buccal film of ZMT has been optimally formulated for buccal delivery showing an efficient enhancing of the rate of drug absorption compared to the oral dosage form. The T_{max} value of ZMT loaded in the buccal film (F4) significantly decreased ($p<0.05$), indicating a quicker onset of action.

3.1. Differential Scanning Calorimetry

In Figure 1, the outcomes of the pure ZMT, unaltered film, and chosen films (F1 and F3) are illustrated through differential scanning calorimetry (DSC). The unaltered film displayed a wide spectrum spanning 25°C to 100°C, reminiscent of the DSC profile observed in numerous prior investigations on CMC (24).

Unprocessed pure ZMT showed an endothermic peak at 140 ºC indicating the melting of ZMT and converting it to a liquid state (25). Upon comparison of the DSC results between the unprocessed ZMT and medicated films (F1 and F3), the drug's thermal behavior has been changed. Specifically, the endothermic peak of the drug, which completely disappeared in the medicated films. This finding may suggest that the drug is in its amorphous state and homogenously dispersed within the film matrix confirming the enhanced ZMT solubility with efficient amorphization effect. After drug dispersion in the polymeric matrix, there is no difference between the thermogram of the plain and drug-loaded films indicating that ZMT has no negative influence on the remaining ingredients of the mucoadhesive buccal film (26).

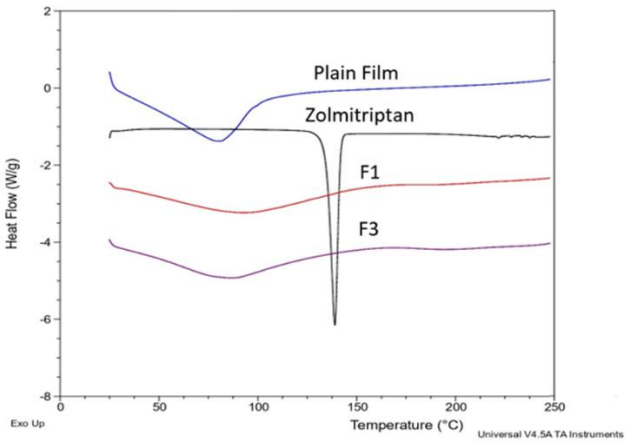

Figure 1. DSC scanning of the pure drug, placebo film, and selected films (F1 and F3)

3.2. Evaluation of the physical characteristics of ZMT-loaded buccal films

The films underwent an evaluation of their physical attributes, encompassing thickness, surface pH, weight consistency, folding resilience, and uniformity in drug content. The results were collated and showcased in Table 2, with the metrics recorded for every 1cm² of the fabricated buccal films, except for the assessment of the effective drug content. The measurements of the films were determined under the assumption that each film contains 2.5 mg of the drug.

Table 2. Various evaluation parameters of ZMT mucoadhesive buccal film.

Formulat-ion	Thickness (mm)	Weight variation (mg)	Surface PH	*ADC (mg/Film)	**F. Endur-ance
Plain	0.075±0.005	10.1±0.25	6.48± 0.03	----	
F1	0.124±0.006	12.1±0.60	6.70± 0.05	2.46±0.16	
F2	0.125±0.013	12.3±0.89	6.45± 0.12	2.38±0.19	>50
F3	0.136±0.018	12.8±0.41	6.80± 0.03	2.38±0.21	
F4	0.157±0.028	14.2±1.05	6.65± 0.08	2.44±0.19	

*ADC: Actual drug content; **F. endurance: Folding endurance.

3.2.1. Visual inspection, microenvironment PH, thickness, and weight uniformity

The physical characteristics of the buccal films were meticulously assessed to investigate the quality and uniformity of the film formulation. The ZMT mucoadhesive buccal films underwent careful visual scrutiny, displaying attributes of optical transparency, consistency, and pliability. Its surface presents a smooth and homogenous texture, free from imperfections such as air entrapment. The film's optical

clarity is pristine and devoid of coloration. Furthermore, it exhibited easy detachment from its petri dish, affirming its suitability for pharmaceutical applications.

The films displayed thickness variations ranging from 0.075±0.005 to 0.157±0.028 mm, with corresponding weight fluctuations spanning 10.1±0.25 to 14.2±1.05 mg. Both film thickness and weight exhibited a positive correlation with drug content, indicating the potential impact of the drug on the films' physical characteristics. As the drug loading increased from 0.6 to 2.5 mg/cm², a non-significant elevation in the buccal film thickness was observed ($p>0.05$), facilitating more practical administration at higher drug loads. The initial lower dose of ZMT allows for gradual dose escalation without impeding buccal administration. Additionally, there was a noteworthy increase in weight uniformity across one cm² of distinct films with a significant rise ($p<0.05$) correlated with the theoretical drug content. Through the judicious selection of the polymer and its proportion in developing buccal films, the surface pH has been maintained as closely aligned with the buccal/salivary pH.

3.2.2. Folding endurance

The folding endurance test results indicate that both the plain and medicated films exhibit optimal flexibility, allowing them to withstand numerous folds, exceeding 50 instances. Ultimately, this pliability is crucial, especially in the context of buccal administration, a feature provided by CMC and PG in the mucoadhesive buccal films due to their film-forming properties. For example, they were employed to formulate films containing diltiazem (27).

3.2.3. Moisture properties

The assessment of moisture uptake in buccal films is an essential *in vitro* characterization that significantly impacts the elastic properties of the prepared films in addition to their mucoadhesive property. The results, depicted in Figure 2, indicated that the film's weight gain follows an exponential manner. The weight gain of the plain polymer has a significant weight increase due to the moisture uptake compared to the medicated films. The weight gain as a result of moisture uptake could be arranged as the following: plain film > F1 > F2 > F3 > F4. That is, maybe, due to the hydrophobic nature of ZMT, it is classified as a class II drug (28,29). This explanation agrees with the finding that an increase in the drug weight led to a decrease in the weight of the hygroscopic medicated film due to decreasing the film moisture uptake. That suggests lower moisture uptake could be achieved with higher drug concentration since the plain film shows the highest increase in weight (30).

3.2.4. Swelling Index

Hydration is the initial step necessary for the swelling of the mucoadhesive polymer to create a suitable macromolecular mesh of adequate size, induce agility in the polymer chains, and improve the intertwining process among polymer and mucin. The influence of ZMT on the swelling behavior of the mucoadhesive polymer (CMC) was also examined, and the results are represented in Figure 3. From the figure, it can be noticed that in the first 20 minutes, there is increasing in the swelling index value of all films. The comparative swelling index rate was in the following order Plain > F1 > F2 > F3 > F4. After 20 minutes the swelling index of all films decreased drastically. Increasing the drug concentration significantly reduced the swelling index of the buccal film ($p<0.01$) compared to plain buccal film. This could be attributed to the poor aqueous solubility of ZMT (31), these results matched with the data obtained from the moisture uptake experiment.

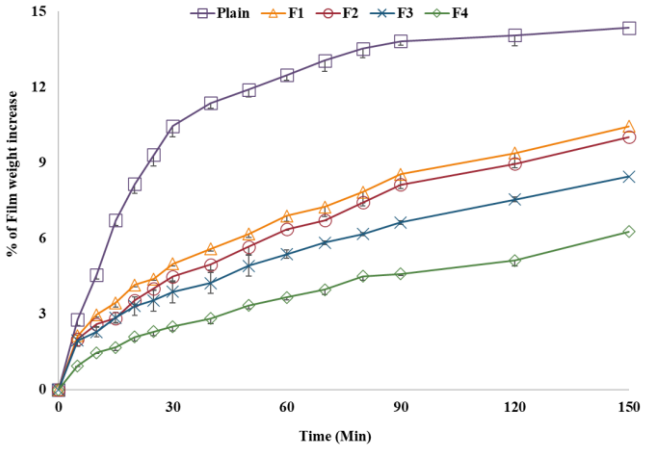

Figure 2. Percentage of film weight's increase after moisture uptake at room temperature and humidity at different time intervals over 150 min (Data represented as mean of triplicates - SD for data clarity).

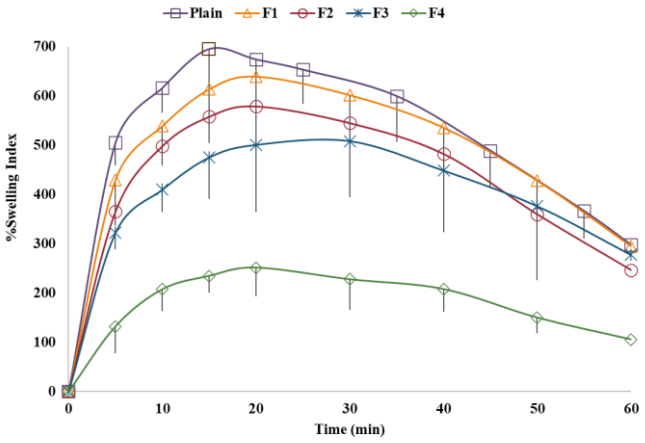

Figure 3. The Plain and medicated mucoadhesive buccal film's swelling index loaded with different drug concentrations. (Data represented as the mean of triplicates - SD for data clarity).

3.2.5. In vitro adhesion Force

Table 3 presents the outcomes of the mucosal adhesion force, adhesion strength, and bond strength tests.

Desirable adhesion properties exhibited by the plain buccal film, which verifies that CMC is a suitable polymer for optimal mucoadhesion characterization. That is due to the adhesion mechanism of CMC (it binds to the mucin molecules on the mucosal surfaces). This interaction is primarily due to the existence of functional groups such as carboxyl (-COOH) in the CMC structure that can result in the formation of hydrogen bonds with the mucin glycoproteins in the mucous layer. These bonds contribute to the adhesive forces that hold the formulation in tight contact with the mucosa (32). As a result, CMC's adhesive properties extend the residence time of the drug delivery system at the application site ensuring prolonged and controlled drug dissolution. However, elevating the drug loading leads to a significant ($p<0.05$) decline in the adhesion characteristics, including adhesion strength, adhesion force, and bond strength for all formulations F1- F4. The observed decrease in adhesive properties with increasing drug concentration can be attributed to several factors. ZMT may alter the properties of the film, including its flexibility and adhesive properties, leading to reduced adhesion. Higher drug concentrations might disrupt the interaction between the film and the mucosal surface resulting in hindering adhesion. ZMT might mask or occupy the adhesive sites on the CMC polymer that are responsible for mucoadhesion. This interference could weaken the ability of the film to bind with the mucosal layer (33).

Table 3. Bioadhesion strength, adhesion force, and adhesion time of the different prepared mucoadhesive buccal films.

Formula	Bioadhesion Strength (g)	Adhesion Force (N)	Bond Strength (NM^2)
Plain	48.3±3.93	0.474±0.0387	0.474±0.0387
F1	38.7±1.94	0.378±0.017	0.378±0.017
F2	35.2±1.37	0.345±0.014	0.345±0.014
F3	32.7±2.08	0.359±0.018	0.359±0.018
F4	27.7±2.52	0.268±0.023	0.268±0.023

3.2.6. In vitro ZMT dissolution study

The formulation of ZMT as a buccal film composed of carboxymethylcellulose (CMC) and propylene glycol (PG) would enhance the aqueous solubility of ZMT and hence offer better *in vitro* dissolution patterns. The combined solubilizing action of CMC and PG has been previously investigated to enhance the bioavailability of several candidates such as simvastatin (34). Figure 4 demonstrates the dissolution pattern of ZMT from various buccal films prepared with consistent drug content (2.5 mg/film). The observed dissolution profile follows the order: of F2 > F4 > F3 > F1, with a notable rise in the amount of drug released for F2 during the initial 20 minutes. In addition, in each case, there is a complete drug release after a certain time.

The disturbance in the drug release profile order could be attributed to the following origin: the release profile is affected by the drug-to-polymer ratio, which is variable in each formula regarding the different dimensions of the film segment loaded with 2.5 mg of ZMT. Herein, the controlling factors are highly interfering including the poor ZMT solubility and different composing amounts of polymeric matrices resulting in diverse film's swellability during the *in vitro* dissolution process. So, the ZMT release behavior is not displayed regularly for 45 minutes.

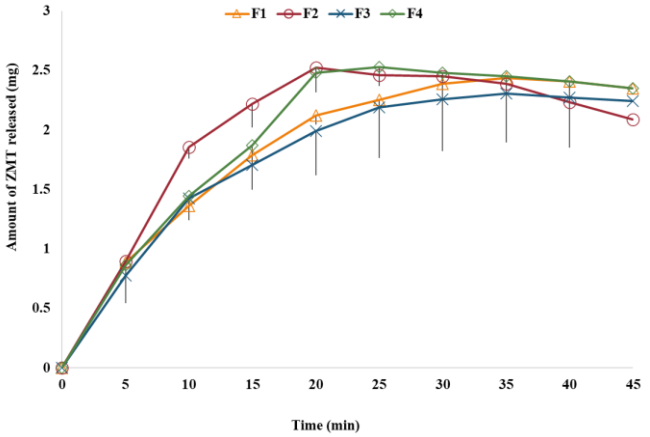

Figure 4. Drug release profile from different mucoadhesive buccal films loaded with various drug concentrations. (Data represented as the mean of triplicates -SD for data clarity).

3.3. Bioavailability study (in Humans):

The in vivo study was conducted to affirm the success of the ZMT muco-adhesive film with enhanced release characteristics. An informed consent form was signed by six subjects after that they were admitted and randomly allocated. The *in vivo* study has been completed with no volunteers' withdrawal. The calibration curve of ZMT in human plasma ranged from 3 ng/ml to 100 ng/ml, the recovered concentrations ranged from 3.61 ± 0.56 ng/ml to 101.19 ± 1.17 ng/ml, corresponding to the percent recovery ranging from 96.33% to 120.43%. The coefficients of variation (%CV) for these recoveries ranged from 1.16% to 15.54%, indicating consistent and reliable results across the various spiked concentrations and high % recoveries of ZMT could be practically found in the plasma samples after drug spiking. Sample preparation utilizing the protein precipitation technique was selected as it is considered the most commonly used bio-analysis sample extraction strategy (35). The spectrofluorometric method of analysis is characterized by a high degree of specificity as it is based on two wavelengths (emission and excitation) which enables the determination of the drug in the presence of other related compounds without interference (36,37).

During the two phases of the study adverse effects were not observed. The mean pharmacokinetic parameters of ZMT are illustrated in Table 4. The mean time plasma concentration curve of the six volunteers for ZMT after the intake of test buccal film versus the reference Amigrawest 2.5 mg oral tablet as illustrated in Figure 5. The high computed value of the standard deviation of the ZMT concentration/time profile, as well as C_{max}, $AUC_{0\rightarrow t}$, and $AUC_{0\rightarrow \alpha}$, were used to indicate the rapid first-step hepatic

metabolism of ZMT, which accounts for its large inter-individual variability.

The *in vivo* investigation showed a substantial difference in the Tmax concerning the treatment, which suggested ZMT was absorbed more quickly than with traditional tablets. While the logarithmic transformed C_{max} and $AUC_{0\rightarrow t}$ values showed a non-significant difference concerning period, sequence, treatment, and subject within the sequence. On the other hand, the log-transformed values of $AUC_{0\rightarrow \alpha}$ results showed a significant difference ($p>0.05$) between subjects within the sequence, reflecting the inter-subject variation while non-significant differences were observed concerning sequence, period, and treatment. The test formulation was created to achieve a rapid onset of action. The results revealed a significant enhancement in the rate of absorption; however, the extent of absorption was not affected.

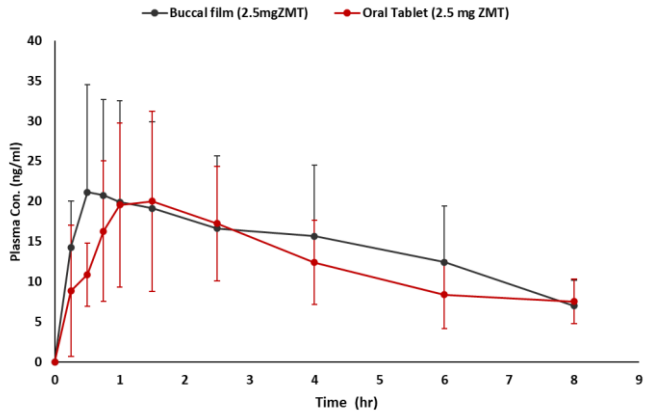

Figure 5. Mean plasma concentration of ZMT following administration of Buccal film (2.5 mg) and oral Tablet (2.5mg) to healthy human volunteers (Mean ±SD)

Table 4. Mean pharmacokinetic parameters of zolmitriptan after administration of reference (Amigrawest, Western pharmaceutical industries 2.5 mg oral) and test (2.5 mg buccal film) after administration to 6 healthy male volunteers (mean ±SD)

	t_{max}	C_{max}	$AUC_{0\rightarrow t}$	$AUC_{0\rightarrow \alpha}$	R.B.%
Ref.	1.33 (±0.60)	21.12 (±11.25)	89.98 (±47.69)	120.42 (±57.05)	126.99
Test	0.63 (±0.26) *	25.32 (±13.61)	114.08 (±63.8)	152.92 (±85.23)	

R.B. % = Relative bioavailability %

* Statistically significant difference.

〖AUC〗_(0→t) is the area under the concentration curve from time zero to time t(8 hr), AUC0–∞ is the area under the concentration curve from time zero to infinity; C_max the peak plasma concentration of ZMT.

ZMT has ideal physicochemical features for buccal delivery, including a low molecular weight (287.36g/mol), water solubility (~200 µg/ml), and moderate lipophilicity (log P = 1.6). ZMT has a positive charge at physiological pH and absorbs in a pH-dependent manner, according to the tertiary amine's pKa (9.55). Furthermore, zolmitriptan oral treatment for acute migraine often produces a sluggish and variable Tmax (2-4 h), delaying the onset of action and reducing its clinical efficacy. The complete ionization of ZMT in stomach pH is probably the cause of its delayed and sluggish absorption in oral therapy (38). Mucoadhesive oral films (MOF) are promising dosage forms that attach to and transport drugs through the buccal mucosa, providing various benefits. These include bypassing the hepatic first-pass effect, rapid commencement of action, and ease of transport and handling. The use of such a dose form is advantageous for medications with low oral bioavailability and those requiring quick absorption (39).

Considering our study, the results demonstrated the success of the buccal film in enhancing the rate of absorption of ZMT in comparison to conventional tablets, especially since ZMT is classified as a class II drug (40).

. Our results revealed the success of buccal film to enhance the rate of absorption only while the extent was not affected. Several studies suggested that ZMT is likely metabolized by CYP1A2 as well as monoamine oxides. The non-significant changes in the extent of ZMT absorption could be related to the presence of a metabolizing enzyme in the buccal cavity as well (41). In addition, ZMT is a substrate to efflux protein transporters P-glycoprotein (P-gp) in the buccal cavity, which could further explain the non-significant enhancement of the extent of ZMT absorption (42).

4. CONCLUSION

Our research has successfully developed a mucoadhesive buccal film for the rapid and efficient treatment of migraine attacks. The formulation, comprising zolmitriptan, exhibited promising results in terms of moisture uptake, thickness, weight uniformity, actual drug content, folding endurance, swelling index, and *in vitro* dissolution. The film's ability to provide enhanced drug dissolution is of particular significance in the context of treating migraine attacks as evidenced by the pharmacokinetic study of the drug in humans. These findings highlight the potential of our mucoadhesive buccal film as a valuable addition to migraine treatment options.

ACKNOWLEDGMENTS

The authors express gratitude to the individuals affiliated with the Pharmaceutical Technology Department at the University of Tanta. Recognition is extended to the volunteers for their participation, endurance, and cooperation throughout the execution of the in-vivo study.

FUNDING

No funding has been received for our study from any governmental, commercial, or non-profit entity.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to the submitted work.

5. REFERENCES

1. Cady RK, Farmer K. Acute and preventative treatment of episodic migraine. In: Headache and Migraine Biology and Management. Elsevier; 2015. p. 69–97.
2. Johnston MM, Rapoport AM. Triptans for the management of migraine. Drugs. 2010;70:1505–18.
3. Sandri G, Ruggeri M, Rossi S, Bonferoni MC, Vigani B, Ferrari F. Chapter 8 - (Trans)buccal drug delivery. In: Martins JP, Santos HA, editors. Nanotechnology for Oral Drug Delivery [Internet]. Academic Press; 2020. p. 225–50. Available from: https://www.sciencedirect.com/science/article/pii/B9780128180389000132
4. Tuchman M, Abu-Shakra S, Silberstein SD. Oral zolmitriptan exhibits high efficacy and low recurrence rates in the acute treatment of true menstrual migraine: results of a randomized controlled trial [abstract no. OR8]. Headache. 2003;43:512.
5. AstraZeneca. Zomig® and Zomig RapimeltTM product information [Internet]. 2022 Nov. Available from: https://www.astrazeneca.ca/content/dam/az-ca/downloads/productinformation/zomig-product-monograph-en.pdf
6. Lionetto L, Casolla B, Mastropietri F, D'Alonzo L, Negro A, Simmaco M, et al. Pharmacokinetic evaluation of zolmitriptan for the treatment of migraines. Expert Opin Drug Metab Toxicol. 2012 Aug 5;8(8):1043–50.
7. Shipp L, Liu F, Kerai-Varsani L, Okwuosa TC. Buccal films: A review of therapeutic opportunities, formulations & relevant evaluation approaches. Journal of Controlled Release. 2022;352:1071–92.
8. Nair AB, Shah J, Jacob S, Al-Dhubiab BE, Patel V, Sreeharsha N, et al. Development of mucoadhesive buccal film for rizatriptan: In vitro and in vivo evaluation. Pharmaceutics. 2021;13(5):728.
9. Yildir E, Sjöholm E, Preis M, Trivedi P, Trygg J, Fardim P, et al. Investigation of dissolved cellulose in development of buccal discs for oromucosal drug delivery. Pharm Dev Technol. 2018;23(5):520–9.
10. Rasool BKA, Khan SA. In vitro evaluation of miconazole mucoadhesive buccal films. Int J Appl Pharm. 2010;2(4):23–6.
11. Jukanti R, Dandu R, Harshita KN, Jayaveera KN. Development of mouth dissolving films of zolmitriptan using pullulan as a film-forming agent. Int J Pharmtech Res. 2012;4(1):83–91.
12. Patil AR, Patil VR,, Deshmukh PK. Formulation and evaluation of mucoadhesive films of zolmitriptan. . Journal of Drug Delivery Science and Technology. 2017;39:155–61.
13. RODRÍGUEZ-GONZÁLEZ F, GUZMÁN-VILLANUEVA D,, VÉLAZ I. GRAVIMETRIC ANALYSIS FOR THE DETERMINATION OF THE WEIGHT UNIFORMITY OF ORAL THIN FILMS. J Pharm Anal. 2018;8(3):177–82.
14. Al-Nemrawi NK, Alzoubi KH, Abualhasan MN,, Alsayyad JJ. Development and characterization of fast dissolving oral films containing rizatriptan benzoate. Drug Development and Industrial Pharmacy Year: 2015. 2015;41(8):1381–9.
15. Alves TFR, Rios AC, da Silva Pontes K, Portella DL, Aranha N, Severino P, et al. Bilayer mucoadhesive buccal film for mucosal ulcers treatment: Development, characterization, and single study case. Pharmaceutics. 2020;12(7):657.
16. Mady O, Hussien S, Abdelkader DH, El-Dahaby E. Metoclopramide loaded buccal films for potential treatment of migraine symptoms: in vitro and in vivo study. Pharm Dev Technol [Internet]. 2023 Jul 3;1–10. Available from: https://doi.org/10.1080/10837450.2023.2231076
17. Alanazi FK, Abdel Rahman AA, Mahrous GM, Alsarra IA. Formulation and physicochemical characterisation of buccoadhesive films containing ketorolac. J Drug Deliv Sci Technol. 2007;17(3):183–92.
18. Habib F, Abdel Azeem M, Fetih G, Safwat M. Mucoadhesive buccal patches of lornoxicam: I-development and in-vitro characterization. Bulletin of Pharmaceutical Sciences Assiut. 2010;33(1):59–68.
19. Nair AB, Al-Dhubiab BE, Shah J, Jacob S, Saraiya V, Attimarad M, et al. Mucoadhesive buccal film of almotriptan improved therapeutic delivery in rabbit model. Saudi pharmaceutical journal. 2020;28(2):201–9.
20. Stone J. Chapter 3 - Sample preparation techniques for mass spectrometry in the clinical laboratory. In: Nair H, Clarke W, editors. Mass Spectrometry for the Clinical Laboratory [Internet]. San Diego: Academic Press; 2017. p. 37–62. Available from: https://www.sciencedirect.com/science/article/pii/B9780128008713000031
21. French D. Chapter Five - Advances in Clinical Mass Spectrometry. In: Makowski GS, editor. Advances in Clinical Chemistry [Internet]. Elsevier; 2017. p. 153–98. Available from: https://www.sciencedirect.com/science/article/pii/S0065242316300828
22. Kong R. 17 - LC/MS Application in High-Throughput ADME Screen. In: Ahuja S, Dong MW, editors. Separation Science and Technology [Internet]. Academic Press; 2005. p. 413–46. Available from: https://www.sciencedirect.com/science/article/pii/S0149639505800613
23. Saka C. Review of analytical methods for identification and determination of triptans. Crit Rev Anal Chem. 2009;39(1):32–42.

24. Mirzaei-Mohkam A, Garavand F, Dehnad D, Keramat J, Nasirpour A. Physical, mechanical, thermal and structural characteristics of nanoencapsulated vitamin E loaded carboxymethyl cellulose films. Prog Org Coat. 2020;138:105383.
25. Chowdhury S, Mudgal J, Arora S, et al. Differential scanning calorimetry studies on zolmitriptan: a selective serotonin receptor agonist. J Therm Anal Calorim. 2013;111(3):1771–7.
26. Grabowska B, Żymankowska-Kumon S, Cukrowicz S, Kaczmarska K, Bobrowski A, Tyliszczak B. Thermoanalytical tests (TG–DTG–DSC, Py-GC/MS) of foundry binders on the example of polymer composition of poly (acrylic acid)–sodium carboxymethylcellulose. J Therm Anal Calorim. 2019;138:4427–36.
27. Latif S, Kokab A, Afzal H, Hameed M, Manzoor M, Batool N, et al. Formulation and characterization of orodispersible film containing diltiazem hydrochloride with taste masked effects. Pak J Pharm Sci. 2022;35(4).
28. Selleck Chemicals. Zolmitriptan Data sheet [Internet]. [cited 2024 Aug 18]. Available from: https://www.selleckchem.com/datasheet/Zolmitriptan(Zomig)-S164902-DataSheet.html
29. Drugs. Zolmitriptan Prescribing Information [Internet]. 2024 [cited 2024 Aug 18]. Available from: https://www.drugs.com/pro/zolmitriptan.html
30. Singh S, Jain S, Muthu MS, Tiwari S, Tilak R. Preparation and evaluation of buccal bioadhesive films containing clotrimazole. AAPS PharmSciTech. 2008;9:660–7.
31. Cayman Chemical. Zolmitriptan Product Information [Internet]. 2022 Oct [cited 2024 Aug 18]. Available from: https://cdn.caymanchem.com/cdn/insert/15871.pdf
32. Javanbakht S, Shaabani A. Carboxymethyl cellulose-based oral delivery systems. Int J Biol Macromol. 2019;133:21–9.
33. Ahmed Kassem A. Formulation approaches of triptans for management of migraine. Curr Drug Deliv. 2016;13(6):882–98.
34. El-Maghraby GM, Abdelzaher MM. Formulation and evaluation of simvastatin buccal film. J Appl Pharm Sci. 2015;5(4):70–7.
35. Castro-Perez J, Prakash C. Recent advances in mass spectrometric and other analytical techniques for the identification of drug metabolites. Identification and Quantification of Drugs, Metabolites, Drug Metabolizing Enzymes, and Transporters. 2020;39–71.
36. Chatwal GR. Instrumental methods of chemical analysis. Himalaya publishing house; 2022.
37. Skoog DA, Holler FJ, Crouch SR. Instrumental analysis. Vol. 47. Brooks/Cole, Cengage Learning Belmont; 2007.
38. Kumria R, Al-Dhubiab BE, Shah J, Nair AB. Formulation and evaluation of chitosan-based buccal bioadhesive films of zolmitriptan. J Pharm Innov. 2018;13:133–43.
39. Silva BMA, Borges AF, Silva C, Coelho JFJ, Simões S. Mucoadhesive oral films: the potential for unmet needs. Int J Pharm. 2015;494(1):537–51.
40. Awadeen RH, Boughdady MF, Meshali MM. Quality by design approach for preparation of zolmitriptan/chitosan nanostructured lipid carrier particles–formulation and pharmacodynamic assessment. Int J Nanomedicine. 2020;8553–68.
41. Drug Bank online. Zolmitriptan Drug Profile [Internet]. [cited 2024 Nov 8]. Available from: https://go.drugbank.com/drugs/DB00315
42. Thorn CF, Aklillu E, Klein TE, Altman RB. PharmGKB summary: very important pharmacogene information for CYP1A2. Pharmacogenet Genomics. 2012;22(1):73–7.