Research Paper

Bioenhanced sublingual tablet of drug with limited permeability using novel surfactant binder and microencapsulated polysorbate: In vitro/in vivo evaluation

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ABSTRACT

Formulation of sublingual tablets of drugs with limited permeability poses a great challenge due to their poor absorption. In this study, bioenhanced sublingual tablets (BESTs) of zolmitriptan were prepared using novel surfactant binder (Pluronic® p123/Sylloid® mixture) to enhance tablet disintegration and dissolution. Microencapsulated polysorbate 80 (Sepitrap™ 80) were included in the composition of BESTs to enhance the drug transport through the sublingual mucosa. Tablets were evaluated for in vitro/in vivo disintegration, in vitro dissolution and ex vivo permeation. Solubility studies confirmed that phosphate buffer; pH 6.8 could be used as dissolution medium for sublingual tablets of zolmitriptan. BEST-5 containing Pluronic® p123/Sylloid® mixture and Sepitrap™ 80 exhibited the shortest in vitro/in vivo disintegration times (<30 s), the highest dissolution at early time dissolution points and the highest enhancement of drug transport through mucosal membrane. The in vivo pharmacokinetic study using human volunteers showed a significant increase in the rate and extent of sublingual absorption with less variations of T_{max} after sublingual administration of both BEST-5 and Zomig-ZMT ODT. Our results proposed that Pluronic® p123/Sylloid® mixture and Sepitrap™ 80 could be promising for the development of sublingual tablets for rapid onset of action of drugs with limited permeability.

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1. Introduction

The sublingual route usually produces a faster onset of action than oral route of administration. The advantage of the sublingual drug delivery is that the drug can be directly absorbed into systemic circulation avoiding the first pass effect in the liver and gut. Moreover, sublingual mucosa is very thin compared to the intestinal or buccal mucosa and highly vascular allowing excellent drug absorption with a rapid onset of action [1–3]. A rapid onset of therapeutic effect is required in many acute conditions such as angina pectoris (sublingual nitroglycerine), cancer pain (sublingual fentanyl) and acute migraine attacks (oral zolmitriptan). Therefore, there is a growing interest in developing sublingual tablets, films [4] or sprays [5] for the treatment of these acute conditions. The development of sublingual tablets, for rapid onset of action, of drugs having limited membrane permeability represents a major challenge. Zolmitriptan is selected as a model drug of limited permeability attacks, with and without an aura, and cluster headaches. It has a selective action on serotonin receptors and is very effective in reducing migraine symptoms. The absolute bioavailability of zolmitriptan is up to 40% for both oral and nasal dosage forms [6,7]. The current oral zolmitriptan tablets present several drawbacks, such as slow onset of action, [8] low bioavailability and large inter-subject variability in the rate of absorption (T_{max}). In addition, migraine attacks delay gastric emptying resulting into delayed absorption during attacks. Therefore, sublingual zolmitriptan could provide fast-acting, non-invasive delivery for migraine attacks [9]. However, the sublingual administration of zolmitriptan poses a great challenge due to its low membrane permeability. It was reported that zolmitriptan has low permeability, prolonged absorption down the gastrointestinal tract [10,11] and its transport is mediated by both passive diffusion and active transporters such as P-gp. The efflux of zolmitriptan by P-gp might play a role in limiting the absorption of zolmitriptan [12]. Numerous research articles describe the preparation of zolmitriptan as sublingual or orally disintegrating tablets [9,13,14]. To our knowledge, no reported trials were so far adopted to overcome the limited membrane permeability of zolmitriptan and enhance the absorption through the sublingual mucosa. To reach such criteria, the
excipients used should ensure rapid disintegration of the tablets to enhance the dissolution of the drug. Moreover, a bioenhancer should be incorporated in the tablet matrix to increase the permeability of the drug through the sublingual mucosa.

Pluronic® p123 and Pluronic® F68 are nonionic block copolymer surfactants widely used as emulsifying agents, solubilizing agents, wetting agents and as tablet binders and coatings [15]. Due to their hydrophilicity (high HLb), Pluronic® were supposed to act as binders without affecting the tablet disintegration. Sepitrap™ 80 is a novel microencapsulated solubilizer (MES) presented as free flowing powder with very small particle size (<200 μm). It contains 45–65% of liquid polysorbate 80 adsorbed onto Alumino silicates carrier (35–55%). Sepitrap™ 80 improves the properties of polysorbate 80 through enhancing its solubilizing and bioenhancing properties due to the large surface area and by allowing incorporation of large amount of polysorbate 80 into tablets (up to 10%).

In this study, bioenhanced sublingual tablets (BEST) of zolmitriptan were prepared by wet granulation technique using novel surfactant binders (Pluronic® p123/Syloid®C) and a bioenhancer such as Sepitrap™ 80. The effects of formulation variables on the disintegration and dissolution behavior of tablets as well as the permeation of drug through mucous membrane were studied. Furthermore zolmitriptan pharmacokinetics was studied after sublingual administration of the developed BEST to human volunteers compared to the market product (Zomig-ZMT ODT).

2. Materials and methods

2.1. Materials

Zolmitriptan was kindly supplied by Amoun Pharmaceutical Company, El-Obour (Egypt). Pluronic® p123, Pluronic® F68, polysorbate 80, Acetoni trile and monobasic sodium phosphate were purchased from Sigma Aldrich Chemical Co., (St. Louis, USA.). Sepitrap™ 80 (microencapsulated solubilizer) was obtained from Seppic S.A. (France). Syloid® 244FP silica was obtained from W.R. Grace & Co.-Conn. (USA). Polyethylene glycol 400 was purchased from El-Nasr pharmaceutical chemicals Co., (Egypt). Pearlitol flash was supplied from Roquette (France). Croscarmellose Sodium was obtained from FMC BioPolymer (USA). Orange flavor, sucralose was kindly supplied from Marcyl Pharmaceutical Industries (Egypt). Distilled water was used throughout the study. All other chemical reagents and solvents were of analytical grade and used as received.

2.2. Determination of equilibrium solubility of zolmitriptan in different media

A known excess amount (50 mg) of drug was shaken with 20 ml distilled water, 0.1 N HCl, PH 1.2 and phosphate buffer, PH 6.8 in an amber-colored glass vials and then left in a thermostatically controlled shaking water bath set at 37 °C for 48 h. The supernatant was filtered through a Millipore filter (pore size 0.45 μm). The filtrate was immediately analyzed by a validated HPLC method using UV detection at 227 nm and C18 column (4.6 mm × 250 mm, 5 μm). The mobile phase composed of acetoni trile:phosphate buffer containing 0.04 M monobasic sodium phosphate solution with PH adjusted to 7.6 with sodium hydroxide solution (50:50, by volume) and the flow rate was 1 mL/min. All experiments were carried out in triplicate. The test results are presented as mean value ± SD.

2.3. Study of the impact of surfactant binders on the in vitro dissolution of zolmitriptan

In vitro dissolution of zolmitriptan was performed to study the effect of surfactant binders on the extent of drug dissolution. Six grams of physical mixtures (PM) of drug and Pluronic® p123 or Pluronic® F68 in a ratio of 1:2 by weight was prepared by mixing the drug and binder in a glass mortar for 5 min. Six grams of PM of drug, Pluronic® p123 and Syloid®C in a ratio of 1:2:2.8 by weight was also prepared by the same method. The dissolution of drug from an amount of PM equivalent to 2.5 mg drug was performed in 500 mL phosphate buffer pH 6.8 maintained at 37 ± 0.5 °C using the USP Dissolution Tester Apparatus II (VK 700, Vankel, USA), at a rotation speed of 50 rpm. Aliquots from the dissolution medium were withdrawn at 1, 2.5, 5, 7.5 and 10 min time intervals. The samples were replaced with fresh dissolution medium of same quantity in order to maintain the volume in the vessel constant. Samples were filtered using 0.45 μm Millipore filter and the drug concentration in the samples was determined using a validated HPLC method as previously mentioned.

2.4. Preparation of bioenhanced sublingual tablet (BEST) of zolmitriptan

Surfactant binder was dissolved in ethyl alcohol and then the drug was well dispersed in alcoholic binder solution. Pearlitol flash was granulated with the binder solution and then dried at 50 °C. The dried blend was mixed with Syloid®C (in case of Pluronic® p123) and sieved through 1 mm mesh screen. The granules were then mixed with the remaining ingredients. Tablets were directly compressed by a single punch tableting machine equipped with 6 mm punch and die set. The compression force and mass of all tablets were kept constant. Batches of 100 bi convex tablets (65 mg each), containing 2.5 mg of zolmitriptan per tablet, were prepared and exposed to further investigation. The composition of BEST is presented in Table 1.

2.5. Evaluation of the prepared BEST

2.5.1. Physical characterization

BESTs were evaluated by carrying out tests for friability, hardness and weight variation. Friability and weight variation were carried out according to the compendial specifications (USP 36) [16].

2.5.2. Drug content uniformity

The prepared tablet was crushed and dispersed in 400 ml distilled water in volumetric flask 500 mL. The dispersion was sonicated for 10 min and then filtered using 0.45 μm Millipore filter. 5 mL of the filtrate was suitably diluted with distilled water and

<table>
<thead>
<tr>
<th>Ingredients (mg)</th>
<th>BEST-1</th>
<th>BEST-2</th>
<th>BEST-3</th>
<th>BEST-4</th>
<th>BEST-5</th>
</tr>
</thead>
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<tr>
<td>Zolmitriptan</td>
<td>2.5</td>
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<td>2.5</td>
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<tr>
<td>Pluronic® F68</td>
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<td>49.5</td>
<td>41.85</td>
<td>40.68</td>
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<tr>
<td>Syloid® 244 FP</td>
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<tr>
<td>Sucralose</td>
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<tr>
<td>Croscarmellose sodium</td>
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<tr>
<td>Magnesium Stearate</td>
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<tr>
<td>Polysorbate 80</td>
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<td>–</td>
<td>0.65</td>
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<tr>
<td>Sepitrap® 80</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>9.1</td>
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<td>81.67</td>
<td>81.67</td>
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</tr>
</tbody>
</table>
The zolmitriptan content was determined using a validated HPLC method as previously mentioned.

2.5.3. In vitro disintegration study
The in vitro disintegration test was carried out on six tablets in phosphate buffer pH 6.8 at 37 ± 0.5 °C using the USP dissolution apparatus (Logan instruments incorporation, NJ, USA). Results are presented as mean value ± SD.

2.5.4. In vivo oral study
In vivo disintegration study was carried out in four healthy volunteers. The volunteers were informed of the purpose and protocol of the study. The protocol was reviewed and approved by the Ethics Review Committee of Faculty of Pharmacy, Cairo University, Egypt. The volunteers were asked to rinse their mouth with 200 ml water. The BEST was placed under the tongue and immediately a stopwatch was started as soon as the tablet contacts the tongue. The in vitro disintegration time was recorded as the time from placing the tablet under the tongue to the time after the last noticeable mass had disintegrated [9]. The swallowing of saliva was prohibited during the test. Taste evaluation was based on the mouth feel and the taste acceptance by the patients.

2.5.5. In vitro dissolution study
The dissolution of drug from BESTs was performed in 500 mL phosphate buffer pH 6.8 maintained at 37 ± 0.5 °C using the USP Dissolution Tester Apparatus II, at a rotation speed of 50 rpm [17] (http://www.accessdata.fda.gov/scripts/cder/dissolution/index.cfm). Aliquots from the dissolution medium were withdrawn at 1, 2.5, 5, 7.5, 10 and 15 min time intervals. The samples were replaced with fresh dissolution medium of same quantity in order to maintain the volume in the vessel constant. Samples were filtered using 0.45 μm Millipore filter and drug content was determined using a validated HPLC method as previously mentioned.

2.5.6. Ex vivo permeation study
Ex vivo permeation study was performed using chicken pouch membrane [18]. The Ex vivo permeation study of drug from the BESTs and the market product were performed in a USP dissolution apparatus tester (USP apparatus I) at 37 ± 0.1 °C. One tablet of BEST and the market tablet, all containing 2.5 mg drug were placed in double open-sided glass cylindrical tubes (2 cm in diameter and 5 cm in length, with area = 3.14 cm²) tightly covered from one side with chicken pouch membrane and made water tight by rubber band [19]. One mL of phosphate buffer pH 6.8 was added to the tablet to simulate the effect of salivary fluid. The loaded tubes were attached from the second side to the shafts of the USP dissolution tester apparatus. This assembly represents the donor compartment. The shafts rotated at a speed of 50 rpm in phosphate buffer pH 7.4. The dissolution vessels (receptor compartment) were filled with 250 mL of phosphate buffer pH 7.4. Three milliliter samples were withdrawn periodically at predetermined time intervals of 5, 10, 15, 30, 45, 60, 90, 120 and 240 min and replaced instantly by an equal amount of fresh phosphate buffer pH 7.4 in order to maintain the same volume. The drug concentration was determined by a validated HPLC method as previously mentioned. The ex vivo permeability study was done in duplicates and the cumulative amount of permeated drug (μg/cm²) was plotted as a function of time.

The efficacy of the different bioenhancers was determined by comparing specific permeation parameters of zolmitriptan in the presence or absence of bioenhancer. This ratio was defined as the enhancement factor (EF), which was calculated using the following equation [18,20];

\[ EF = \frac{Q_{\text{bioenhanced}}}{Q_{\text{control}}} \]

where \(Q_{\text{bioenhanced}}\) is cumulative permeated amount of zolmitriptan in the presence of bioenhancer at 15, 60 min and at the end of permeation period (BEST-3, BEST-4 and BEST-5). \(Q_{\text{control}}\) is cumulative permeated amount of zolmitriptan in the absence of bioenhancer at the same permeation intervals (BEST-1).

2.5.7. In vivo pharmacokinetic study in healthy human volunteers

2.5.7.1. Study subjects. The study was carried out to compare the pharmacokinetics of zolmitriptan from the BEST 5 to a market product (Zomig-ZMT® 2.5 mg tablet). A single dose, two-period randomized crossover design was adopted under fasting condition. Four healthy adult male volunteers participated in this comparative study (weight 60–75 kg, age between 30 and 35 years, and height from 167 to 185 cm), and all are nonsmokers. The biochemical examination of the volunteers revealed normal kidney and liver functions. The nature and the purpose of the study were fully explained to them. None of the volunteers were on any drug treatment one week before the participation in the study. An informed written consent was obtained from each volunteer. The in vivo study protocol was reviewed and approved by the research ethics committee (REC) at the Faculty of Pharmacy, Cairo University, Egypt (REC number is PI (710)). The protocol complies with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for humans.

2.5.7.2. Study design. The study was performed on two periods. Period I, half the number of volunteers (group 1) received BEST-5 containing 2.5 mg (treatment A). The other half (group 2) received one orally disintegrating tablet Zomig®-ZMT (treatment B). The tablets were inserted sublingually and positioned with the tablet surfaces in contact with the ventral tongue and the floor of the mouth. In period II, group 1 received treatment B and group 2 received treatment A. Both treatments were administered after 12-h overnight fasting. Food and drink (other than water, which was allowed after 2 h) were not allowed until 4 h after dosing, and then a standard breakfast and lunch were given to all volunteers according to a time schedule. A washout period of 1 week separated the periods.

2.5.7.3. Sample collection. Blood samples (4 mL) were drawn into evacuated heparinized glass tubes through an indwelling cannula at the following sampling times: 0 min (pre-dose), 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10 and 12 h after the administration of each treatment. Blood samples were centrifuged at 4000 rpm for 15 min at 4 °C; plasma was transferred directly into plastic tubes and stored frozen at −20 °C for drug analysis.

2.5.7.4. Sample preparation. All frozen human plasma samples were thawed at ambient temperature. Human plasma samples (0.5 mL) were placed in 7 mL glass tubes, and 100 μL of internal standard (IS) solution (Rizatriptan benzoate, 20 ng/mL in methanol) and 100 μL of 1 M NaOH were added to each and vortexed for 30 s. Four mL Methyl t-butyl-ether was then added, and samples were then vortexed for 5 min. The tubes were then centrifuged for 10 min at 3000 rpm. The upper organic phases were then transferred to clean glass tubes, filtered through 0.45 μm Millipore filter and evaporated to dryness using centrifugal vacuum concentrator (Eppendorf, Germany) at 45 °C. Dry residues were then reconstituted with 150 μL of mobile phase and 7.5 μL was injected into an UPLC–MS/MS system using the autosampler.

2.5.7.5. Quantitative determination of zolmitriptan in plasma. The quantitative determination of zolmitriptan in human plasma was performed by modified UPLC–MS/MS procedures described by Mahmoud et al. [9]. The mobile phase consisted of acetonitrile,
2.5.7.6. Pharmacokinetic analysis. Non-compartmental pharmacokinetic analysis was performed using computer program, Kinetica\textsuperscript{®} (version 5, Thermo Fischer Scientific, NY, USA). The $t_{1/2}$ (h) was calculated as 0.693/K. The maximum drug concentration ($C_{\text{max}}$, ng/mL) and the time to reach $C_{\text{max}}$ ($t_{\text{max}}$, h) were obtained from the individual plasma concentration-time curves. The area under the curve AUC$_{0-12}$ (ng h/mL) was calculated as AUC$_{0-\infty} = AUC_{0-12} + C_t/K$ where $C_t$ is the last measured concentration at the time $t$.

2.5.7.7. Statistical analysis. The pharmacokinetic parameters $C_{\text{max}}$, AUC$_{0-12}$, and AUC$_{0-\infty}$ were compared between treatments A and B with ANOVA test for the untransformed data and calculating 90% confidence interval of the ratio of test/reference using log-transformed data. The inclusion of the confidence interval within 0.8–1.25 was taken as a demonstration of bioequivalence [21]. The nonparametric Kruskal-Wallis test was used to compare the medians of $t_{\text{max}}$ for the treatments A and B. The untransformed values for $t_{1/2}$ were compared with the ANOVA test. The level of significance was $\alpha = 0.05$. A $p$ value of $<0.05$ was considered statistically significant. The sample size ($n = 4$) was selected not based on statistical consideration but rather on an economic consideration.

3. Results and discussion

3.1. Selection of the dissolution medium

The solubility of zolmitriptan was determined in water, phosphate buffer, pH 6.8 (simulated salivary fluid) and 0.1 N HCl, pH 1.2 (simulated gastric fluid) at 37 °C. Several researches mentioned different values of water solubility of zolmitriptan ranging from 1.3 to 20 mg/mL [9,10]. Our solubility determinations showed that the solubility of zolmitriptan in water is 1.70 ± 0.16 mg/mL. The solubility of zolmitriptan was found to be pH dependent. The maximum drug solubility (20.26 ± 2.90 mg/mL) was obtained in 0.1 N HCl which agrees with the previous researches [9]. The solubility of zolmitriptan in phosphate buffer, pH 6.8 was found to be 9.79 ± 0.68 mg/mL which is about 6 times its solubility in water. FDA recommends 500 mL of 0.1 N HCl as dissolution medium for orally disintegrating tablets of zolmitriptan. However, in our research work, it was preferred to use 500 mL of phosphate buffer, pH 6.8 as dissolution medium for the bioenhanced sublingual tablets due to two reasons. First: the high solubility of drug in this medium which maintains sink conditions. Second: phosphate buffer, pH 6.8 is considered as simulated salivary fluid so it was more suitable to be used as dissolution medium for sublingual tablets.

3.2. Study of the impact of surfactant binder on the in vitro dissolution of zolmitriptan

Two types of surfactant binders (Pluronic\textsuperscript{®} P123 and Pluronic\textsuperscript{®} F68) were used for the formulation of BEST of zolmitriptan. It was expected that surfactant binders had a dual function as they bind the powder together forming the granules and enhance the compression into tablets. Moreover, they increase dissolution by increasing hydrophilicity of tablet matrix and promoting solubilization of active ingredient [22,23]. The effect of both types of Pluronics\textsuperscript{®} on the dissolution of zolmitriptan was evaluated by performing in vitro dissolution of PM of drug and each Pluronic\textsuperscript{®} (1:2, w/w) in 500 mL phosphate buffer, pH 6.8. As shown in Fig. 1, in the absence of surfactant binder, the drug displayed complete dissolution after 7.5 min. PM of Pluronic\textsuperscript{®} F68 and Pluronic\textsuperscript{®} P123 gave different dissolution profiles of zolmitriptan. Although complete dissolution of drug was obtained from PM of Pluronic\textsuperscript{®} F68 after 5 min, only 53.45 ± 2.47% of zolmitriptan was dissolved from PM of Pluronic\textsuperscript{®} P123 after 10 min. It was reported that Pluronic\textsuperscript{®} F68 is a hydrophilic nonionic triblock copolymer with surface active properties. It is widely used in pharmaceutical formulations as an emulsifying or solubilizing agent [22,24,25]. The lower percentage dissolved of zolmitriptan from PM of Pluronic\textsuperscript{®} P123 could be explained based on its lower hydrophilicity (HLB ~ 12) and its physical form (paste) [26] (http://worldaccount.basf.com). The paste like consistency of Pluronic\textsuperscript{®} P123 limits the penetration of aqueous fluid into the drug particles resulting in retardation of drug dissolution. PM of drug, Pluronic\textsuperscript{®} P123 and Syloid\textsuperscript{®} (1:2.2.8, w/w) was prepared to convert the semisolid nature of Pluronic\textsuperscript{®} P123 into free flowing powder. Syloid\textsuperscript{®} FP silica is a highly porous, micronized silica powder. When added to a formulation, the high porosity is capable of adsorbing a considerable amount of liquid, keeping the product dry and free flowing and increases the surface area of the drug exposed to dissolution medium [27] (https://grace.com/pharma-and-biotech/en-us/Documents/Syloid/M417_Syloid%20Anti-Caking%20App%20NoteFINAL.pdf). As expected, the PM of drug, Pluronic\textsuperscript{®} P123 and Syloid\textsuperscript{®} displayed complete dissolution of the drug after only 2.5 min (Fig. 1). Thus, Pluronic\textsuperscript{®} F68 or Pluronic\textsuperscript{®} P123 and Syloid\textsuperscript{®} mixture could be used as surfactant binder in the developed BEST of zolmitriptan.

3.3. Physical characteristics of BEST of zolmitriptan

The weight of BESTs ranged from 64.8 ± 1.2 to 65.9 ± 0.9 mg. The percentage friability for all the BESTs was below 1%, indicating that the friability is within the compendial limits (USP 36). In all the formulations, the hardness test indicated good mechanical strength and the percentage of drug content was between 99.8% and 101.2%.

3.4. In vitro disintegration

Fig. 2 shows the in vitro disintegration times of BEST of zolmitriptan. Although there is no stated specification regarding the in vitro disintegration tests of sublingual tablets, USP 36 contains four monographs of sublingual tablets with different disintegration times ranging from 2 min (for nitroglycerin and isosorbide
dinitrate sublingual tablets) to 15 min (for ergoloid mesylates sublingual tablets) [16]. The disintegration time of orally disintegrating tablets is preferred to be one minute or less [9,16]. The objective of this work was to develop BEST of zolmitriptan having disintegration time less than one min, preferably 30 s or less. The in vitro disintegration times of BEST-1, BEST-3, BEST-4 and BEST-5 are 11.1 ± 0.92, 15.2 ± 2.50, 14.3 ± 2.41 and 22.3 ± 1.52 s, respectively. However, the in vitro disintegration time of BEST-2 is 139.4 ± 5.60 s (Fig. 2). It is obvious that BEST-2 containing Pluronic® F68 as hydrophilic surfactant binder had the longest disintegration time (>2 min). This observation conforms well to the results obtained by Kaul et al. [23]. It was proposed that Pluronic® F68 may act as a binding agent or promote the binding action of the binder by improving wetting of the active ingredient and excipient blend within the tablet, which results in larger and denser granules which retard the penetration of aqueous medium. This leads to prolongation of disintegration time and retardation of early time point dissolution [23]. The relatively long disintegration time of BEST-5 could be due to the presence of lower amount of Gellan gum®. The short disintegration times of BEST containing Pluronic® p123/Syloid® mixture as surfactant binder (<30 s) could be explained based on the highly porous nature of Syloid® which coats the surface of the other ingredients to reduce adherence and aggregation and allows capillary wetting of granules for better disintegration.

3.5. In vivo oral study

Fig. 2 shows the in vivo disintegration times of BESTs of zolmitriptan which are in accordance with the in vitro disintegration results. The in vivo disintegration times of BEST-1, BEST-3, BEST-4 and BEST-5 are 13.2 ± 1.20, 19.4 ± 1.20, 16.8 ± 2.20 and 26.4 ± 2.61 s, respectively. However, the in vivo disintegration time of BEST-2 is 159.6 ± 10.10 s (Fig. 2). It is obvious that BEST-2 containing Pluronic® F68 as surfactant binder displayed the lowest dissolution where only 15.65 ± 0.78 and 34.84 ± 1.95% were dissolved after 1 and 2.5 min, respectively. The percentage dissolved of drug from BEST-1, having the same composition as BEST-2 with Pluronic® p123/Syloid® mixture as surfactant binder, was 31.73 ± 2.36 and 48.6 ± 2.26% after 1 and 2.5 min, respectively. These dissolution results are in accordance with the in vitro in vivo disintegration results where faster disintegrating BEST (<30 s) gave higher initial dissolution percentage. Incorporation of polysorbate 80 (0.65 mg) in the composition of sublingual tablets (BEST-3) had no effect on the initial dissolution of the drug where 31.05 ± 0.77 and 47.7 ± 2.68% were dissolved after 1 and 2.5 min, respectively. BEST-4 and BEST-5 containing Sepitrap® 80 (equivalent to 1 and 5 mg polysorbate 80) displayed the highest initial dissolution percentage. The percentage dissolved of drug from BEST-5 after 1 and 2.5 min was 60.15 ± 3.56 and 71.95 ± 2.98%, respectively. The relatively long disintegration time of BEST-5 (22.3 ± 1.52 s) did not affect the rapid dissolution of drug from tablet. This observation indicates that the drug dissolution is not only disintegration limited but depends also on the solubilizing effect of polysorbate 80 which would be subsequently released from Sepitrap® 80. Microencapsulated polysorbate 80 (Sepitrap® 80) allow incorporation of larger amount of polysorbate in the tablet matrix due to its solid nature. Polysorbate 80 is a commonly used non-ionic surfactant which enhances solubility and dissolution of poorly soluble drugs [28]. The dissolution test was repeated in 0.1N HCl as dissolution medium to confirm the validity of phosphate buffer pH 6.8 to discriminate between the dissolution rate of zolmitriptan from all BEST formulations. Similar to the dissolution results in phosphate buffer pH 6.8, the dissolution of zolmitriptan from BESTs in 0.1 N HCl can be arranged in the same order but with higher dissolution values. The percentage dissolved of drug from BEST-5, BEST-4, BEST-1, BEST-3 and BEST-2 after 2.5 min was 80.12 ± 1.78, 73.32 ± 2.10, 54.28 ± 2.41, 56.81 ± 1.94 and 46.14 ± 1.77%, respectively.

3.6. In vitro dissolution study

Fig. 3 shows the in vitro dissolution profiles of BEST of zolmitriptan in phosphate buffer pH 6.8. As shown, all the BESTs show complete dissolution of the drug at 15 min and their dissolution profiles differ in the early time points of dissolution (1 and 2.5 min). BEST-2 containing Pluronic® F68 as surfactant binder displayed the lowest dissolution where only 15.65 ± 0.78 and 34.84 ± 1.95% were dissolved after 1 and 2.5 min, respectively. The percentage dissolved of drug from BEST-1, having the same composition as BEST-2 with Pluronic® p123/Syloid® mixture as surfactant binder, was 31.73 ± 2.36 and 48.6 ± 2.26% after 1 and 2.5 min, respectively. These dissolution results are in accordance with the in vitro in vivo disintegration results where faster disintegrating BEST (<30 s) gave higher initial dissolution percentage. Incorporation of polysorbate 80 (0.65 mg) in the composition of sublingual tablets (BEST-3) had no effect on the initial dissolution of the drug where 31.05 ± 0.77 and 47.7 ± 2.68% were dissolved after 1 and 2.5 min, respectively. BEST-4 and BEST-5 containing Sepitrap® 80 (equivalent to 1 and 5 mg polysorbate 80) displayed the highest initial dissolution percentage. The percentage dissolved of drug from BEST-5 after 1 and 2.5 min was 60.15 ± 3.56 and 71.95 ± 2.98%, respectively. The relatively long disintegration time of BEST-5 (22.3 ± 1.52 s) did not affect the rapid dissolution of drug from tablet. This observation indicates that the drug dissolution is not only disintegration limited but depends also on the solubilizing effect of polysorbate 80 which would be subsequently released from Sepitrap® 80. Microencapsulated polysorbate 80 (Sepitrap® 80) allow incorporation of larger amount of polysorbate in the tablet matrix due to its solid nature. Polysorbate 80 is a commonly used non-ionic surfactant which enhances solubility and dissolution of poorly soluble drugs [28]. The dissolution test was repeated in 0.1N HCl as dissolution medium to confirm the validity of phosphate buffer pH 6.8 to discriminate between the dissolution rate of zolmitriptan from all BEST formulations. Similar to the dissolution results in phosphate buffer pH 6.8, the dissolution of zolmitriptan from BESTs in 0.1 N HCl can be arranged in the same order but with higher dissolution values. The percentage dissolved of drug from BEST-5, BEST-4, BEST-1, BEST-3 and BEST-2 after 2.5 min was 80.12 ± 1.78, 73.32 ± 2.10, 54.28 ± 2.41, 56.81 ± 1.94 and 46.14 ± 1.77%, respectively.

3.7. Ex vivo permeation study

Based on the results of in vitro in vivo disintegration and in vitro dissolution tests, BEST-2 was excluded from further ex vivo permeability study as it showed the longest disintegration times and the lowest percentage dissolved at the early time points of dissolution. Fig. 4 shows the permeation profiles of sublingual tablets of zolmitriptan containing polysorbate 80 (BEST-3) and Sepitrap® 80 (BEST-4 and BEST-5) as bioenhancers compared with
that of Market product (Zomig-ZMT™ orally disintegrating tablets) through chicken pouch membrane. The permeation profile of sublingual tablets containing no bioenhancer (BEST-1) was used as a control. It is obvious that the drug permeated was higher from sublingual tablets containing Sepitrap™ 80 than that from tablets containing polysorbate 80. BEST-5 exhibited the highest drug permeation compared to control tablet at different permeation time intervals (15, 60 and 240 min). The EF of BEST-3 and market product after 15, 60 and 240 min was approximately 1 indicating that no enhancement of drug absorption through membrane which may be due to no bioenhancer in the formula composition (the market product) or due to lower amount of bioenhancer in the tablet composition (0.65 mg) as in the case of BEST-3. The EFs of BEST-4 and BEST-5 after 15, 60 and 240 min were (1.60, 1.22 and 1.50) and (1.9, 1.39 and 2.01), respectively. The higher permeation of drug from sublingual tablets containing Sepitrap™ 80 may be due to drug solubilization mechanism or membrane interaction mechanism or both. As previously mentioned, all the BESTs showed complete dissolution of the drug at 15 min confirming that the drug was completely free for absorption after 15 min. Thus, the increase of drug permeation by Sepitrap™ 80 was through the interaction of microencapsulated polysorbate 80 with the chicken membrane. The enhancement of drug permeation by polysorbate 80 through buccal mucous membranes was previously reported [29–32]. Sepitrap™ 80 improves the properties of polysorbate 80 as bioenhancer not only through improving membrane interaction properties and maximizing its solubilization properties due to the large surface area but also by allowing incorporation of large amount of polysorbate 80 into tablets (up 10%).

3.8. In vivo pharmacokinetic study

BEST-5 exhibited the shortest in vitro/in vivo disintegration times (<30 s) and faster in vitro dissolution rate (about 72% after 2.5 min). Moreover, it showed optimum bioenhanced absorption through buccal membrane due to the inclusion of Sepitrap™ 80 in its composition. Therefore BEST-5 was selected for the in vivo pharmacokinetic study compared to Zomig-ZMT 2.5 mg orally disintegrating tablets.

The mean plasma zolmitriptan concentration versus time curves following sublingual administration of BEST-5 and Zomig-ZMT to four volunteers is shown in Fig. 6. The mean pharmacokinetic characteristics are summarized in Table 2. The mean C_{max} estimated from BEST of zolmitriptan (2.45 ± 0.24 ng/mL) was larger and statistically significantly different (p = 0.02115) relative to the mean from the marketed product (1.82 ± 0.14 ng/mL) and the 90% confidence interval for the test/reference mean ratio of the log-transformed data of C_{max} (1.16–1.47) failed to satisfy the bioequivalence criteria. The nonparametric test, Kruskal-Wallis test, was used to compare the medians of T_{max} of both test and market products confirming that T_{max} of bioenhanced sublingual tablet is smaller and statistically significant different from that of market product. The significant higher value of C_{max} and the lower values of T_{max} of BEST-5 compared to that of the market product confirm the enhanced absorption of drug from the sublingual mucosa due to the inclusion of Sepitrap™ 80 as a bioenhancer. The rapid absorption of drug from BEST-5 correlates well with the results of ex vivo permeation which showed that zolmitriptan exhibited higher permeation from bioenhanced sublingual tablets than from the market tablet.

It was reported in the literature that individual plasma zolmitriptan profiles after oral administration show double peaks in some volunteers and, because of this, the time to reach C_{max} (T_{max}) may vary between 0.5 and 6 h and T_{max} could be at the time of the first or second peak [33–35]. The double peaks are not observed following intravenous (IV) doses of zolmitriptan, suggesting that this phenomenon is due to the prolonged absorption down the gastrointestinal tract rather than enterohepatic recirculation [11]. These findings confirm that zolmitriptan is a BCS class-III drug (highly soluble and poorly permeable) [10]. On the other hand, the
sublingual absorption of zolmitriptan from BEST-5 and market tablet showed less variation in $T_{max}$ (1–1.5 and 1.5–2.5, respectively) which may be due to the rapid transport across the sublingual mucosa which is five times thinner than buccal mucosa and more obviously due to the bioenhancing effect of Sepitrap™ 80 in BEST-5 on the sublingual mucosa.

Statistically significant differences ($p < 0.05$) were found between the pharmacokinetic parameters AUC$_{0–12}$ and AUC$_{0–\infty}$ determined for both BEST-5 and the market tablet. The 90% confidence intervals for the test/reference mean ratio of the log-transformed data of AUC$_{0–12}$ and AUC$_{0–\infty}$ are (1.18–1.62) and (1.17–1.60), respectively. Thus, the 90% confidence interval cannot satisfy bioequivalence. There was no significant difference ($p > 0.05$) between the tested formulations in terms of $t_{max}$ where its value complies with that reported in the literature [11]. Based on these findings, it could be concluded that a promising BEST of zolmitriptan was successfully designed. However, because of the small number of subjects recruited in the study, the results can only be considered preliminary, and further studies with a larger number of subjects should be conducted to prove the clinical usability of the developed bioenhanced sublingual formulation.

4. Conclusion

In this study, bioenhanced sublingual tablets of zolmitriptan containing Pluronic® p123/Syloid® mixture as surfactant binder demonstrated fast disintegration (<30 s) and dissolution. Incorporation of Sepitrap™ 80 not only increased the initial disso- lution but also bioenhanced the absorption of drug through the sublingual mucosa. The increase of the sublingual absorption of drug by Sepitrap™ 80 was through the interaction of microencapsulated polysorbate 80 with the sublingual mucosa rather than affecting drug dissolution. The in vivo pharmacokinetic study suggests that the BEST developed in this work may be useful for the management of migraine attacks due to the significant increase in the rate and extent of sublingual absorption with more uniform absorption patterns (less variability of $T_{max}$). Further clinical studies with a larger number of patients should be performed to prove the clinical efficacy of the bioenhanced sublingual tablet in migraine attack.

References


Table 2

<table>
<thead>
<tr>
<th>BEST-5</th>
<th>Zomig-ZMT</th>
<th>Statistical test</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (ng/mL)</td>
<td>2.45 ± 0.24</td>
<td>1.82 ± 0.14</td>
</tr>
<tr>
<td>AUC$_{0–12}$ (ng h/mL)</td>
<td>8.99 ± 1.05</td>
<td>6.48 ± 0.8</td>
</tr>
<tr>
<td>AUC$_{0–\infty}$ (ng h/mL)</td>
<td>9.3 ± 1.07</td>
<td>6.97 ± 0.77</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>2.13 ± 0.25</td>
<td>2.41 ± 0.43</td>
</tr>
<tr>
<td>$T_{max}$ (h) $^a$</td>
<td>1–1.5</td>
<td>1.5–2.5</td>
</tr>
</tbody>
</table>

$^a$ Median. $^b$ Range.