Zaleplon loaded bi-layered chronopatch: A novel buccal chronodelivery approach to overcome circadian rhythm related sleep disorder

Michael M. Farag⁎, Nevine S. Abd El Malak, Soad A. Yehia
Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Egypt

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ABSTRACT

The aim of this study was to develop a novel buccal bi-layered chronopatch capable of eliciting pulsatile release pattern of drugs treating diseases with circadian rhythm related manifestation. Zaleplon (ZLP) was used as a model drug intended to induce sleep and to treat middle of night insomnia. The chronopatch was prepared adopting double casting technique. The first layer was composed of a controlled release patch containing ZLP-Precirol melt granules intended to release ZLP in a sustained manner to maintain sleep and to prevent early morning awakening. The second layer was composed of a fast release lyophilized buccal disc containing ZLP loaded SNEDDS (Z-SNEDDS) intended for rapid sleep induction. Pharmacokinetic parameters of ZLP from the chronopatch were compared to those of the immediate release capsule, Siesta®, as reference in Mongrel dogs using a randomized crossover design. The appearance of two peaks having two Cmax and Tmax proved the pulsatile release pattern. The increase in relative bioavailability of ZLP from the chronopatch was 2.63 folds. The results revealed the ability of the developed ZLP loaded bi-layered chronopatch to be a candidate for overcoming early morning awakening without middle of night dose administration.

1. Introduction

The concept of ideal drug delivery has changed significantly over the last few decades, this was mainly due to a better understanding of the underlying causes and pathophysiological factors related to the diseases. Therefore making the drug immediately available following administration or constant drug delivery will not provide such idealized delivery in many cases (Wilson and Crowley, 2011). A shifted paradigm namely ‘chronodelivery’ has emerged adopting the concept of adapting drug delivery to the body’s circadian rhythm.

The relation between many body functions as well as many diseases with the circadian rhythm is well established. Therefore in order to maximize the therapeutic outcome, it is better to adjust the maximum drug release to coincide the manifestation of clinical symptoms based on circadian timing. This could preferably be used for the management of diseases with time dependant manifestation such as bronchial asthma, myocardial infarction, rheumatic disease, angina pectoris and sleep disorders (Maroni et al., 2010).

Many successful devices applied the concept of chronodelivery, among them we could mention ChronoCap® adopting injection molding of hydroxypropyl cellulose to obtain swellable/erodible shells, capable of drug release after a required lag time (Gazzaniga et al., 2009). While the ChronoCap® was based on hydrophilic release-controlling polymer, the Time-Clock® system was depending on an erodible layer composed of natural waxes and surfactants (Pozzi et al., 1994). Another device is Pulsincap™ which depended on a release plug composed of cross-linked PEG 8000 hydrogel, the lag phase was determined by the time needed for plug removal (Stevens et al., 2002). It is worthy to mention that none of the above systems was intended for the buccal route which make them unsuitable for highly metabolized drugs, which highlight the need for a novel device such as the bi-layered chronopatch intended for the buccal route to bypass the first pass hepatic metabolism.

Sleep is a primary need for all human beings just as food and shelter, being in a stressful world people may occasionally face psychological pressures distorting their biological clock leading to unsatisfactory sleeping pattern. Almost 25% of adults may suffer from insomnia, 10% of cases become chronic (Summers et al., 2006). Insomnia can be classified into four main types: frequent awakening, early morning awakenings, poor sleep quality and difficulty in falling asleep (Cricco et al., 2001).

Zaleplon (ZLP) is a non-benzodiazepine hypnotic used for short term management of insomnia mainly for sleep induction (Dooley and Plosker, 2000), it has a great affinity to α1 subunit located on GABA_A receptor in the brain, therefore it enhances the action of GABA more selectively than benzodiazepines. ZLP suffers mainly from extensive hepatic first pass metabolism leaving only 30% systemically available.
and short elimination half-life (1 h) (Drover, 2004). Also it has shown efficacy in treatment of middle of night insomnia without hangover effects (Verster et al., 2004). Formulation of ZLP into buccal bi-layered chronopatch could avoid these problems. Literature search revealed that no previous studies were carried out to formulate bi-layered chronopatch containing ZLP.

The aim of this study was to formulate ZLP into bi-layered chronopatch to allow the pulsatile release of ZLP through the buccal route in order to avoid its extensive hepatic metabolism, enhance its bioavailability and prevent early morning awakening. This work mainly focus on the preparation and characterization of the second layer consisting of the lyophilized buccal disc containing ZLP loaded SNEDDS, also the preparation, characterization and in vivo evaluation of the chronopatch to estimate the pharmacokinetics of the drug following buccal administration of the chronopatch to Mongrel dogs compared to marketed product.

2. Materials and methods

2.1. Materials

Zaleplon (ZLP) was received as a kind gift from October Pharma, Egypt. Methanol and Acetonitrile (HPLC grade); were purchased from Sigma-Aldrich Co. St. Louis, USA. Capryol™ 90 [Propylene Glycol Monocaprylate (Type II)]; Labrafitt® M 1944 CS (Oleyl Polyoxy-6 Glycerides NF); Labrafac® PG (Propylene Glycol Dicaprylate/Dicaprate); Labrasol® (Caprylocaproyl Polyoxyglycerides); Transcutol® HP (Diethylenglycol Monoethyl Ether), Lauroglycol® 90 [Propylene Glycol Monolaurate (Type II)], were received as a kind gift from Gattefosse Co., St-Priest, France. Polyethylene Glycol (PEG) 200, was purchased from Fluka, Switzerland. Cremophore® RH 40 (Polyoxyethylene 40 Hydrogenated Castor Oil USP/NF), Cremophore® EL (Polyoxyethylene 35 Hydrogenated Castor Oil USP/NF), Solutol® HS 15 (Polyoxy-5 Glyceryl Laurate USP/NF) were purchased from BASF, Germany. Miglyol® 840 (Propylene glycol dicaprylocaprate), was purchased from Sasol Hamburg, Germany. Syloid® FP 244 (silicone dioxide, NF) was purchased from Grace Davison (Grace GmbH and Co. KG, Germany). Gelatin, El Nasr pharmaceuticals Co., Egypt. Torsemide (internal standard) was supplied by Multi-Apex Pharma, Egypt. Siesta® capsule, containing 10 mg ZLP (AlAndalous, Cairo, Egypt, Batch No.: 140151). All other reagents and chemicals used were of analytical reagent grade.

2.2. Solubility studies of ZLP in different components of self-nanoemulsifying drug delivery systems (SNEDDS)

The equilibrium solubility of ZLP in various oils, surfactants and co-surfactants was determined using the method described by Dixit and Nagarsethen (2008). In brief, known excess amount of the drug (100 mg) was added to 3 mL of the investigated component in a vial and shaken for 72 h at 30 ± 0.5 °C in a thermostatically controlled shaker bath to attain equilibrium. The contents of the vials were then centrifuged at 3000 rpm for 10 min using an ultracentrifuge to precipitate the undissolved ZLP. Aliquots (0.1 mL) from the supernatants were then withdrawn and filtered through a cellulose filter (Millipore® filter 0.22 µm) and measured spectrophotometrically at λmax = 236.6 nm) using UV/VIS spectrophotometer (UV-1601 PC), ( Shimadzu, Kyoto, Japan) after appropriate dilution with methylene chloride (1:5000).

2.3. Construction of ternary phase diagram and effect of drug loading

The selected components -based on the solubility studies- comprising of Capryol™ 90 as the oily phase together with either Cremophore® RH 40 or Cremophore® RH 40:Labrasol® 1:1 as surfactant and Transcutol® HP as co-surfactant. Thirty-six points of self-emulsifying systems were prepared in each phase with varying concentrations of oil, surfactant and co-surfactant till reaching a final concentration of 100% w/w (Abdelbary et al., 2013; Basalious et al., 2010). Phase diagrams were constructed by weighing appropriate amounts of surfactant and co-surfactant first into small vials, mixed by vortex mixing for 5 min, then appropriate amount of oil is then added to prepare 300 mg system.

From each mixture point on the phase diagram, 300 mg was diluted 100 times with distilled water and the mixture was stirred using a magnetic stirrer with a rotation speed of 125 rpm at 37 °C. Visual observation of the diluted nanoemulsions was carried out immediately for investigating self-emulsification ability, phase separation and presence of any precipitate. The diluted nanoemulsions were left for 24 h for stability assessment. Clear or slight bluish dispersions and translucent nanoemulsions were used to draw the ternary phase diagram. The percentage transmittance of each point was measured to confirm the clarity. To determine the effect of drug addition on the nano-emulsion boundary and the self-emulsifying performance of the prepared SNEDDS, the phase diagrams were also constructed in the presence of ZLP with constant drug loading of 5 mg drug in 300 mg system for all the prepared SNEDDS.

2.4. Characterization of the selected Z-SNEDDS

2.4.1. Globule size and polydispersity index (PDI) determination

Z-SNEDDS (1–6) were diluted 100 times with phosphate buffer saline (pH = 6.8) and were subjected to constant stirring on a magnetic stirrer with 125 rpm at 37 °C. Then each sample was placed directly in the module and analyzed by diffraction light scattering at 25 °C and a scattering angle of 173° using Zetasizer Nano ZS (model MAM 5000, Malvern instrument limited, Worcestershire, UK). This experiment was done in triplicates and the average results (± SD) were recorded (Kaur et al., 2013).

2.4.2. Transmission electron microscopy (TEM)

The morphology of (Z-SNEDDS) as a representative sample was investigated using Joel (JEM-1230) transmission electron microscope (Tokyo, Japan) to confirm the morphology and size of globules. Briefly, an aliquot of the prepared system was diluted 100 times with distilled water and filtered with Millipore® filter 0.2 µm, then adequate amount of 1% phosphotungstic acid was added and gently mixed. One drop of the mixture was placed on the carbon-coated grid, drained off the excess and left to dry to be observed under TEM.

2.4.3. Preparation of lyophilized Z-SNEDDS buccal discs (Mahmoud and Salah, 2012)

Lyophilization of liquid Z-SNEDDS is difficult as the components of the isotropic mixture are not volatile. To overcome this problem, the selected SNEDDS were adsorbed to Syloid® FP 244 as a solid carrier with a constant ratio 2:6:1 (w/w) which was selected upon previous preliminary studies where different ratios were studied and was found to be the optimum ratio to obtain free flowing powder. Then, the solidified Z-SNEDDS were mixed with 5% mannitol as a cryoprotectant and dispersed in 3% gelatin solution as inner support. The dispersion was then homogenized for 3 min using SilentCrusher S homogenizer (Heidolph, Germany) at 15000 rpm to ensure homogenized dispersion. The dispersion is then individually casted in a special mold so that a specific weight of dispersion containing the desired dose is casted in each well. The mold is then frozen at −20 °C overnight and then freeze-dried for 24 h using a Novalyphe-NL 500 Freeze-dryer, (Savant Instruments, Halprook, NY).

2.5. Characterization of the prepared lyophilized Z-SNEDDS buccal discs

2.5.1. Weight uniformity

Ten discs, from each formula, were individually weighed and the
mean of discs weight was calculated. Results are presented as mean value ± standard deviation (SD) (European Pharmacopoeia, 2002).

2.5.2. Drug content uniformity

The prepared buccal discs from each batch were dissolved in 50 mL of 10% methanol in water in distilled water and the solution was filtered, suitably diluted and the ZLP content was analyzed spectrophotometrically at 230.2 nm using UV/VIS spectrophotometer (UV-1601 PC), (Shimadzu, Kyoto, Japan) (Abdelbary et al., 2016). This test was done in triplicates.

2.5.3. Friability test

Ten discs, from each formulation, were accurately weighed and placed in the friabilator drum. The discs were rotated at 25 rpm for 4 min and then removed, deducted and re-weighed. The percentage loss in weight was calculated and taken as a measure of friability using the following equation (European Pharmacopoeia, 2002).

\[
\text{weight before} - \text{weight after} \times 100 \over \text{weight before}
\]

2.5.4. In-vitro disintegration time

Disintegration times of the prepared lyophilized buccal discs were determined with six discs in distilled water at 15–25 °C in USP tablet disintegration apparatus (Veego, VTD-D, Mumbai, India). The disintegration time was defined as the time necessary to completely disintegrate until no solid residue remains or only a trace amount of soft residue remains on the screen. All results are presented as mean value ± SD (European Pharmacopoeia, 2002).

2.5.5. In vitro release studies of the prepared lyophilized buccal discs

The release profiles of ZLP from the lyophilized buccal discs were determined in a dissolution tester (Varian VK7000, Cary, NC, USA) following the USP paddle method. All tests were conducted in 250 mL phosphate buffer saline (pH = 6.8). The release medium was maintained at a temperature of 37 ± 0.5 °C with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 5 mg. At definite time intervals, a 3 mL sample was withdrawn and replaced by phosphate buffer saline (pH = 6.8) to maintain sink condition; these samples were assayed spectrophotometrically for ZLP content at 230.2 nm (Abdelbary et al., 2016). The test was done in triplicates.

2.5.6. Scanning electron microscopy

Surface morphology and cross-sections of selected formula FF2 were examined using Jeol JSM-5200 scanning electron microscope (Tokyo, Japan). Cross-section samples were prepared by cutting a thin slice using a scalpel. The samples were fixed on a brass stub using double-sided adhesive tape and then made electrically conductive by coating, in a vacuum, with a thin layer of gold (~150 Å) for 30 s. The micrographs were taken at an excitation voltage of 25 kV.

2.6. Preparation of ZLP loaded bi-layered chronopatch

The statistically optimized formula from the first part of this work (Farag et al., 2017) (composed of Precirol/ZLP ratio of 5:1 equivalent to 5 mg ZLP, HPMC solution/PVA solution weight ratio of 3:1, and 7.5% Ethyl cellulose) was prepared by melt granulation technique followed by emulsification/casting/solvent evaporation technique, this patch represent the first layer providing the delayed pulse.

After complete drying of the first layer, the best achieved lyophilized disc in this investigation containing an equivalent dose of 5 mg of ZLP-representing the second layer that would provide the immediate release pulse- was double casted (Preis et al., 2014) on the first layer and was frozen prior to lyophilization as previously described. The bi-layered chronopatch contained a 10 mg dose of ZLP equally divided between the two layers.

2.7. Characterization of ZLP loaded bi-layered chronopatches

2.7.1. Drug content uniformity

The prepared ZLP loaded bi-layered chronopatches were dissolved in 100 mL of 10% methanol in water in distilled water and tested adopting the same procedure used with the lyophilized buccal discs.

2.7.2. Scanning electron microscopy

Cross-section of the bi-layered chronopatch was examined adopting the same procedure used with the lyophilized buccal discs.

2.8. In-vivo study of the prepared ZLP loaded bi-layered chronopatch

2.8.1. Study design

The study was approved by the Institutional Animal Care and Use Committee (IACUC) Cairo university- Faculty of Science, approval number: CU-III-F-46-17. The design of this study is an open-label, randomized crossover design, two periods, two treatments. Six healthy male Mongrel dogs (20 ± 2.3 kg) were randomly numbered and divided into two dosing groups of equal size. Both group were anesthetized by thiopental I.V. injection (10 mg/kg) (Streisand et al., 1995) for easy application of the patches.

2.8.2. Procedure

On the first period of the study, the first group received one capsule of the market reference product Siesta® containing 10 mg ZLP and the other group received the ZLP loaded bi-layered chronopatch. A washout period of one week separated the two periods of the study then the treatments were switched.

The test treatment was applied to the buccal mucosa of the gingivae without moistening before application by applying light force for 30 s using finger-tip, and they were removed after 12 h. The jaws were kept close for 1 h using a gauze band with adjusted pressure to prevent them from removing the patch, then the bands were removed. The standard treatment was dropped through their throat with 50 mL of water. Food and drink (other than water, which was allowed after 2 h) were not allowed until 12 h after dosing (Kraus et al., 1991).

Three milliliters blood samples for the determination of ZLP were withdrawn from the dogs at the following time intervals at zero (pre-dose), 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9 and 12 h post dose.

2.8.3. Processing of samples

Heparinized blood samples were centrifuged at 4000 rpm for 10 min at 4 °C, and plasma was separated and frozen pending drug analysis. Samples were extracted adopting modified liquid-liquid extraction approach (Maurer et al., 2002).

Briefly, for each 50 μL of internal standard, 0.3 mL Sorenson phosphate buffer (pH = 7.4, 10 mM), 0.3 mL sodium bicarbonate, 0.5 mL plasma and 1 mL diethyl ether:ethyl acetate (50:50) were added, the mixture was vortexed for a half minute and centrifuged (10 min, 4000 rpm). The organic layer was transferred to a vial, 0.15 mL sodium hydroxide was added to the aqueous layer (pH was adjusted to 10) and 1 mL diethyl ether:ethyl acetate (50:50) was added. The mixture was vortexed and centrifuged (10 min, 4000 rpm). The organic layer was combined and evaporated to dryness under N2 at 60 °C. The residue was dissolved in 0.1 mL methanol and used for LC–MS/MS analysis (Montenarh et al., 2014).

Chromatographic separations were performed on a reversed phase Waters SunFire C18 column (2.1 × 150 mm, 3.5 μm). The mobile phase consisted of two solutions: eluent A (10 mM aqueous ammonium formate + 0.1% formic acid) and eluent B (acetonitrile + 0.1% formic acid).
acid). The flow rate was set to 0.5 mL/min. The injection volume was 3 μL. The detection was done using triple quadrupole detector MS/MS. The analysis was operated at the MRM (multiple reaction monitoring) mode (Montenarh et al., 2014).

3. Results and discussion

3.1. Solubility studies of ZLP in different components of self-nanoemulsifying drug delivery systems (SNEDDS)

The equilibrium solubility of ZLP in different SNEDDS components was investigated and recorded in Table 1. Based on the solubility studies two system were selected: Capryol™ 90: Cremophore® RH 40: Transcutol™ HP (system A) and Capryol™ 90: [Cremophore® RH 40: Labrasol® (1:1): Transcutol™ HP (system B).

<table>
<thead>
<tr>
<th>Saturated solubility (mg/ml) ± SD</th>
<th>Capryol™ 90</th>
<th>Lauroglycol™ 90</th>
<th>Miglyol® 840</th>
<th>Labrasol® PG</th>
<th>Surfactant</th>
<th>Labrasol®</th>
<th>Cremophor® RH 40</th>
<th>Solutol® HS 15</th>
<th>Labrafil® 1944M</th>
<th>Cremophor® EL</th>
<th>Co-surfactant</th>
<th>Transcutol™ HP</th>
<th>PEG 200</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19.24 ± 0.352</td>
<td>12.40 ± 0.311</td>
<td>3.12 ± 0.186</td>
<td>2.91 ± 0.214</td>
<td>25.91 ± 0.254</td>
<td>22.45 ± 0.198</td>
<td>21.52 ± 0.224</td>
<td>14.85 ± 0.377</td>
<td>2.96 ± 0.096</td>
<td>42.62 ± 2.036</td>
<td>32.83 ± 1.541</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data are mean values (n = 3) ± S.D.

3.2. Construction of ternary phase diagram and effect of drug loading

Two phase diagrams were constructed for plain SNEDDS showing a nano-emulsification region about 49.65% for (system A) and 33.76% for (system B) as shown in Fig. 1. The different SNEDDS which were visually assessed, on the basis of opaqueness observed and percentage transmittance measured, upon diluting 100 times with phosphate buffer saline (pH = 6.8). Only clear grades [grade (A), transmittance > 95%] and translucent mixtures [grade (B), transmittance > 90%] were selected to determine the effect of drug loading on the nano-emulsion region while other mixtures were rejected.

In this study, there is no change in the nano-emulsion region observed after drug incorporation with phase diagram systems as they exhibited same area before drug addition about 49.65% for (system A) and 33.76% for (system B) as illustrated in Fig. 1.

Generally, efficient emulsification was attained when the S/Cos concentration was 70% w/w or more (Oh et al., 2011). That could be explained by the stabilization of the O/W interface upon increasing the amount of surfactant or co-surfactant leading to the formation of a layer around the emulsion droplets and reducing the interfacial energy as well as providing a mechanical barrier to coalescence. It was also observed that high concentration of oil forms poor emulsion with reduced ability to entrap water upon dilution (Abdelbary et al., 2013).

As the aim was to enhance the solubility of ZLP while ensuring its fast release, the lowest ratio of oil is selected in both systems while varying S/Cos. ratio. Six systems were prepared Z-SNEDDS (1-6) with constant drug loading of 5 mg in each 300 mg system as shown in Table 2.

3.3. Characterization of the selected Z-SNEDDS

3.3.1. Globule size and polydispersity index (PDI) determination

Determination of globule size is important in the formulation of SNEDDS. Small globule size enhance the drug release and hence, the bioavailability (Zhang et al., 2008). Systems with mean globule size below 200 nm fulfil the criteria of SNEDDS (Zhang et al., 2008). Table 2 shows that all the investigated systems possessed a mean globule size < 40 nm. This was attributed to the use of the proper surfactant/co-surfactant mixture which reduced the free energy of the system. Also, the surfactant/co-surfactant mixture provided a strong mechanical barrier to prevent globules coalescence (Nepal et al., 2010). Also, it should be noticed from Table 2 that increasing the surfactant

<table>
<thead>
<tr>
<th>Oil</th>
<th>Surfactant</th>
<th>Co-surfactant</th>
<th>W/O Emulsion Region</th>
<th>Percent Transmittance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Clear</td>
<td>Transparent</td>
<td>49.65%</td>
<td>&gt; 95%</td>
</tr>
<tr>
<td>B</td>
<td>Clear</td>
<td>Transparent</td>
<td>33.76%</td>
<td>&gt; 90%</td>
</tr>
</tbody>
</table>

Fig. 1. A: Plain SNEDDS prepared with Capryol™ 90, Cremophore® RH 40 and Transcutol™ HP (system A), B: Plain SNEDDS prepared with Capryol™ 90, Cremophore® RH 40, Labrasol® and Transcutol™ HP (system B), C: ZLP loaded SNEDDS (system A) and D: ZLP loaded ZSNEDDS (system B).
concentration on the behalf of co-surfactant resulted in decrease of globule size, such a decrease in globule size may be the result of more surfactant being available to decrease the interfacial tension and subsequently stabilization of the oil–water interface. A few studies have reported similar trends of decreased globule size with increased surfactant concentration for various self-emulsifying systems (Nazzal et al., 2002). In addition, from the table it is obvious that the tested formulations possessed PDI values ranging from 0.144 ± 0.006 to 0.292 ± 0.021. All systems with PDI values > 0.3, means the presence of poly dispersed system (wide size distribution) (Verma et al., 2003). As previously stated in different researches, low PDI values mean a more uniform sample which in turn increases its stability for longer periods of time (Lü et al., 2007).

### 3.3.2. Transmission electron microscopy

TEM image of a representative sample Z-SNEDDS2 post distillation with distilled water and filtration was given in Fig. 2 A. Spherical globules were observed with average size of 17.97 ± 0.48 nm. Furthermore, no signs of drug precipitation were observed inferring the stability of the formed nanoemulsion.

### 3.4. Preparation of lyophilized Z-SNEDDS buccal disc

The produced lyophilized buccal discs FF (1–6) were visually assessed for cohesion and physical defects. They were accepted except for formulations FF3 and FF6 as they produced very friable discs which could be attributed to their high surfactant content that imparted high viscosity and prevented the binding of particles despite the presence of gelatin as inner support. Therefore formulations FF3 and FF6 were excluded from the study.

### 3.5. Characterization of the prepared lyophilized Z-SNEDDS buccal disc

#### 3.5.1. Weight uniformity

Results of weight uniformity are represented in Table 3, the presence of insignificant S.D. indicated the homogeneity of the prepared discs.

#### 3.5.2. Drug content uniformity

All discs showed homogenous drug content within permitted limits (± 10%), results are presented as mean percentage ± SD in Table 3.

#### 3.5.3. Friability test

Friability studies revealed that formulated discs weren’t cracked, cleaved, or broken after tumbling and the calculated percentage weight loss was within the acceptable range (less than 1%) and also it indicates the successful role of gelatin as inner support. When aqueous solutions of gelatin are rapidly cooled below 40 °C, a rough 3D gel network with water trapped in the mesh is formed (Djagny et al., 2001). The process of freeze-drying for the frozen gelatin solution causes the trapped frozen water to sublime, leaving behind only the 3D network. This was in accordance to Guan et al. who proved the importance of gelatin as an inner support in the solidification of liposomes by freeze drying technique (Guan et al., 2015). Results of friability test are shown in Table 3.

#### 3.5.4. In vitro disintegration time

The results complied with the British pharmacopoeia regulations for oral lyophilisates which mentioned that the disintegration time should be less than 3 min (British Pharmacopeia, 2012). The mechanism of disintegration is due to weakening of the intermolecular bonds upon penetration of the disintegration medium between the disc’s excipients and consequently resulting in complete disintegration of the discs (Ali Husban et al., 2010). Results for in vitro disintegration time are shown in Table 3.

#### 3.5.5. In vitro release studies of the prepared lyophilized buccal discs

The release profiles of ZLP from the lyophilized buccal discs, drug powder and marketed product Siesta® are illustrated in Fig. 3A. The represented values are the average of three measurements ± S.D. Statistical analysis was done using one-way (ANOVA) to study the effect of varying the surfactant: co-surfactant ratio and type of surfactant system on the percentage of ZLP released after 5 min (Q5min). About 82.33 ± 3.4% to 94.36 ± 1.47% of the drug was released within the first five minutes in contrary to drug powder and Siesta® capsule where only 19.36 ± 0.86% and 23.27 ± 3.14% of ZLP were only released after 5 min respectively.

It should be noted that Siesta® capsule had superior drug release to drug powder as it contains sodium lauryl sulphate surfactant as written in the product leaflet that could enhance the drug release. The percentage of ZLP released from the lyophilized buccal discs was noticeably superior when compared to Siesta® capsule and drug powder, this could indicate that the SNEDDS successfully enhanced the drug release as a result of drug dissolution improvement. The release of ZLP from the prepared lyophilized buccal discs after 5 min was in the following descending order: FF2 (94.36 ± 1.47%) > FF1 (86.5 ± 1.44%) > FF4 (82.33 ± 3.4%) > FF5 (69.21 ± 0.76%). The statistical analysis of Q5min using ANOVA showed that the percentage of ZLP released from formula FF2 consisting of Capryol™ 90:Cremophore® RH 40:Transcutol™ HP (10:40:50) was significantly higher than other formulations (P < 0.05), this might be due to increasing the surfactant ratio on the behalf of co-surfactant could improve the release, this results are in consistence with the findings of self-emulsification time where formula FF2 showed the shortest emulsification time, which could result in better drug solubilization and improved release.

The type of surfactant system was also found to affect the percentage of ZLP released from the prepared lyophilized buccal discs, it was found that systems containing Cremophore® RH 40 only (FF1 and FF2) possessed higher release profile when compared to systems containing Cremophore® RH 40:Labrasol® 1:1 as the surfactant system (FF4 and

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**Table 2**
Composition and characterization of the selected Z-SNEDDS.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Composition</th>
<th>Globule size (nm) ± S.D.</th>
<th>Polydispersity Index (PDI) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-SNEDDS1</td>
<td>10 10 80</td>
<td>39.41 ± 1.23</td>
<td>0.281 ± 0.020</td>
</tr>
<tr>
<td>Z-SNEDDS2</td>
<td>10 40 50</td>
<td>16.89 ± 0.34</td>
<td>0.271 ± 0.027</td>
</tr>
<tr>
<td>Z-SNEDDS3</td>
<td>10 80 10</td>
<td>14.94 ± 0.31</td>
<td>0.144 ± 0.006</td>
</tr>
<tr>
<td>Z-SNEDDS4</td>
<td>10 20 70</td>
<td>24.50 ± 1.08</td>
<td>0.292 ± 0.021</td>
</tr>
<tr>
<td>Z-SNEDDS5</td>
<td>10 40 50</td>
<td>19.13 ± 1.13</td>
<td>0.282 ± 0.068</td>
</tr>
<tr>
<td>Z-SNEDDS6</td>
<td>10 80 10</td>
<td>15.27 ± 0.72</td>
<td>0.231 ± 0.045</td>
</tr>
</tbody>
</table>

* (Capryol® 90),  
* (1–3 Cremophor® RH 40 and 4–6 Cremophor® RH 40: Labrasol® 1:1),  
* (Transcutol® HP).
FF5). This could be explained by the fact that Cremophore® RH 40 had higher self-emulsifying capacity than that of Labrasol®, therefore the addition of latter on the behalf of the former resulted in decreased self-emulsification ability and reduced the release of ZLP. The emulsification ability could be attributed to the chemical structure of the surfactant. When the hydrophobic chain length of the surfactant was equal to that of the oil as in the case of Labrasol (C8) and Capryol™ 90 (C8-10) minimum or no emulsification was noted (Bandivadekar et al., 2013; Shah et al., 1994). This also could explain the reason that there is no significant difference (P = 0.109) between FF1 Capryol™ 90:Cremophore® RH 40:Transcutol™ HP (10:10:80) and FF4 Capryol™ 90:Cremophore® RH 40:Labrasol®:Transcutol™ HP (10:10:10:70), as Labrasol® being of no or minimum emulsification ability will lead to a little or no effect, therefore no significant difference (P > 0.05) was found between them and FF1 has slightly higher release as it has higher Transcutol™ HP content in which the drug has the highest solubility.

Based on the above results, a candidate formula FF2 showing statistically significant (P < 0.05) Q5min = 94.36 ± 1.47% was chosen to be incorporated into bi-layered chronopatch and for in vivo evaluation.

The lyophilized buccal disc FF2 was composed of Capryol™ 90:Cremophore® RH 40:Transcutol™ HP (10:40:50) adsorbed on Syloid® FP 244 with a constant ratio 2.6:1 (w/w) mixed with 5% mannitol as a cryoprotectant and dispersed in 3% gelatin solution as inner support.

### 3.5.6. Scanning electron microscopy

SEM micrographs revealed that the microscopic structure of the surface and cross-section of the lyophilized Z-SNEDDS buccal disc of formula FF2 comprises a homogenous highly porous structure with a pore size range of 33.6–92.7 μm as illustrated in Fig. 2B and C.

### 3.6. Characterization of Zaleplon loaded bi-layered chronopatch

#### 3.6.1. Drug content uniformity

Drug content of the bi-layered chronopatch was done in triplicates, the bi-layered chronopatch showed homogenous drug content about 98.37% ± 3.21% which is within permitted limits (± 10%) (European Pharmacopoeia, 2002).

![Fig. 2. A: TEM of Z-SNEDDS2 after reconstitution with distilled water. B: SEM of formula FF2 in surface views. C: SEM of formula FF2 in cross-section view. D: SEM of ZLP loaded bi-layered chronopatch in cross section views.](image)

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Weight uniformity (mg ± SD)</th>
<th>Drug content uniformity (% w/w ± SD)</th>
<th>Friability test (%)</th>
<th>In-vitro disintegration time (sec ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF1</td>
<td>384.2 ± 0.070</td>
<td>98.40 ± 6.40</td>
<td>0.31</td>
<td>106.5 ± 9.19</td>
</tr>
<tr>
<td>FF2</td>
<td>387.1 ± 0.899</td>
<td>103.98 ± 2.91</td>
<td>0.58</td>
<td>87.5 ± 7.77</td>
</tr>
<tr>
<td>FF4</td>
<td>382.5 ± 0.848</td>
<td>97.16 ± 4.01</td>
<td>0.44</td>
<td>95 ± 5.65</td>
</tr>
<tr>
<td>FF5</td>
<td>385.7 ± 0.212</td>
<td>107 ± 0.79</td>
<td>0.72</td>
<td>125.5 ± 4.94</td>
</tr>
</tbody>
</table>

* The composition of formulations FF(1,2,4 and 5) is the same as Z-SNEDDS(1,2,4 and 5) in Table 2, adsorbed to Syloid® FP 244 with a constant ratio 2.6:1 (w/w), mixed with 5% mannitol as a cryoprotectant and dispersed in 3% gelatin solution as inner support.
3.6.2. Scanning electron microscopy

The cross section SEM micrographs of the bi-layered chronopatch showed that the 2 layers bound. Also the casting interface was not visible as well as there is no connective line between the layers as illustrated in Fig. 2D.

3.6.3. In-vitro drug release

The release profiles of ZLP from bi-layered chronopatch, drug powder and Siesta® in phosphate buffer saline (pH = 6.8) were studied. The release data illustrated in Fig. 3B. The represented values are the average of three measurements ± S.D.

The release profile of the bi-layered chronopatch showed an immediate pulse releasing approximately half the dose 52.14% ± 4.31% during the first hour followed by a controlled release pulse releasing the remaining of the dose. About 101.49% ± 0.59% of drug were released during 12 h compared to drug powder and marketed product where 99.77% ± 0.69% and 100.36% ± 0.542% were released respectively after 4 h. Therefore the bi-layered chronopatch could successfully control ZLP release in a pulsatile manner.

3.7. In-vivo study of the prepared ZLP loaded bi-layered chronopatch

According to the average plasma levels of 6 dogs completing the study as shown in Fig. 3C, the relative bioavailability was found to be
Table 4
Summary of the pharmacokinetic parameters of ZLP following the oral administration of Siesta® 10 mg capsules and the buccal administration of the bi-layered chronopatch.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>ZLP loaded bi-layered chronopatch</th>
<th>Siesta®10 mg capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>81.65 ± 9.47</td>
<td>95.58 ± 10.26</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.0 ± 0</td>
<td>0.5 ± 0.27</td>
</tr>
<tr>
<td>AUC0-t (ng.h/ml)</td>
<td>186.04 ± 49.81</td>
<td>489.85 ± 64.79</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>2.29 ± 0.19</td>
<td>4.06 ± 0.07</td>
</tr>
<tr>
<td>Cmax (1)</td>
<td>95.58 ± 10.26</td>
<td></td>
</tr>
<tr>
<td>Tmax (1)</td>
<td>0.5 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>AUC0-t (2)</td>
<td>489.85 ± 64.79</td>
<td></td>
</tr>
<tr>
<td>MRT (2)</td>
<td>3.67 ± 0.51</td>
<td></td>
</tr>
</tbody>
</table>

263.3% for the tested bi-layered chronopatch, based on the average AUC0-t of the tested formula compared to that of marketed capsule Siesta®. This demonstrates that the bi-layered chronopatch managed to increase the bioavailability of ZLP 2.63 folds in comparison to the oral immediate release marketed capsule. This could be due to the avoidance of the first pass hepatic metabolism by the buccal route. Similar results were previously obtained (Shivanand et al., 2011), where buccal administration of extensively metabolized drugs, resulted in enhancement of their bioavailability.

The estimates of the mean pharmacokinetic parameters of ZLP are given in Table 4. Statistically significant (p < 0.05) differences between the two treatments for Cmax, AUC(0–12), Tmax and MRT were obtained.

Concerning the plasma concentration time curve of the bi-layered chronopatch, the appearance of two peaks having two Cmax and Tmax could prove the pulsatile release pattern which could allow the chronopatch to induce sleep through the fast release pulse and to maintain the sleep without middle of night dose administration through the sustained release pulse.

4. Conclusion
ZLP loaded bi-layered chronopatch was successfully prepared. Pharmacokinetic study in Mongrel dogs showed that the chronopatch was able to release the drug in a pulsatile manner with enhanced bioavailability of ZLP 2.63 folds in comparison to the immediate release marketed capsule Siesta®. Chronopatch could be a promising approach for treatment of diseases affected by circadian rhythm through chronodelivery of drugs especially for those suffering from extensive first pass metabolism and short elimination half-lives. Based on the pharmacokinetic data, the dual pulse could allow sleep induction and maintenance without middle of night dosing which could prevent early morning awakening with possible dose reduction due to enhanced bioavailability.

Conflict of interest
The authors have no conflict of interest to declare.

References
European Pharmacoep. 2002. Published by the Directorate for the Quality of Medicines of the Council of Europe (EDQM). Strasbourg, France.
Farag, M.M., Abd El Malak, N.S., Yehia, S.A., 2017. Controlled buccal patches of Zaleplon using melt granulation technique: an approach to overcome early morning awa-
tile delivery, Annual Meeting & Exposition of the Controlled Release Society.
Guan, P., Lu, Y., Qi, J., Niu, M., Lian, R., Wu, W., 2015. Solidification of liposomes by freeze-drying: the importance of incorporating gelatin as interior support on en-
Kaur, G., Chandel, P., Harikumar, S., 2013. Formulation development of self nanoem-
ulsifying drug delivery system (SNEEDS) of celexobix for improvement of oral bio-
availability. Pharmacopharm 120–133.
Kraus, C., Shaaya, A., Ulmer, J., Hutchings, D., Menon, A., Sakr, A., Ritschel, W.A., 1991. Pharmacokinetics and bioavailability of papaverine HCl following intravenous, per-
Mahlmoud, A.A., Salah, S., 2012. Fast relief from migraine attacks using fast-disin-
oral-cavitation and sonotrode stimulation. Int. J. Pharm. 1–8.
brary-assisted identification and validated quantification of oral antidiabetic of the sulfonamidetype in plasma by atmospheric pressure chemical ionization liquid chromatography–mass spectrometry. J. Chromatogr. B 63–73.