Controlled buccal patches of Zaleplon using melt granulation technique: An approach to overcome early morning awakening

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ABSTRACT

This study deals with the incorporation of melt granules in buccoadhesive patches prepared using polymers of opposing solubilities. Zaleplon (ZLP) is a Non-Benzodiazepine Hypnotic (NBZH) that suffers from extensive first pass hepatic metabolism and very short elimination half-life. The aim of this study was to develop a reliable dosage form that was capable of extending the release of ZLP to avoid early morning awakening so to improve sleep quality and also increase its bioavailability. ZLP was incorporated into Precirol-based melt granules and then further formulated into buccal patches prepared using HPMC, PVA and Ethyl cellulose. A Box-Behnken Design was adopted to statistically optimize the formulation variables, HPMC solution/PVA solution weight ratio, Precirol/ZLP ratio and percentage Ethyl cellulose. Fifteen formulae were prepared and evaluated regarding drug content uniformity, thickness uniformity, moisture loss, water sorption, mucoadhesion strength, surface pH, DSC and in-vitro release. The best achieved formula (composed of 5:1 Precirol:ZLP, 3:1 HPMC:PVA and 7.5% Ethyl cellulose) was able to control the release, where 87.24% of ZLP was released after 12 h and the patch showed acceptable mucoadhesion properties. The results revealed the ability of the developed ZLP buccal patches to be a candidate for overcoming early morning awakening.

1. Introduction

Many people face every now and then psychological tensions, job pressures or emotional disturbances distorting their biological clock leading to unusual sleeping pattern [1]. Insomnia is commonly seen among 30–35% of adults, about 10% of cases become chronic [2]. It can be diagnosed by frequent awakening, early morning awakenings, poor sleep quality and difficulty in falling asleep [3]. It is not just a primary reason for mental impairment but it can impair human effective dealing with every day’s activity even it can compromise the immune system [4]. Major depressive disorders e.g. bipolar depression is associated with difficulty in maintaining sleep, early morning awakenings and poor sleep quality [5]. Unsurprisingly it can be associated with increased mortality [6].

Zaleplon (ZLP) is a NBZH indicated for the short term – 2 to 4 weeks-management of insomnia [7], it binds mainly to α1 subunit located on GABAA receptor in the brain. It enhances the action of GABA more selectively than benzodiazepines. ZLP undergoes an extensive hepatic first pass metabolism leaving only 30% systemically available, its terminal elimination half-life is 1 h so it is mainly used for sleep induction [8]. Also it has shown efficacy in treatment of middle of night insomnia without hangover effects [9].

Since the birth of pharmaceutical sciences, an emerging challenge has been raised for the formulation of drugs suffering from extensive first pass metabolism into more bioavailable form. Buccal delivery has always been an appealing choice as it offers a shortcut to systemic circulation, bypassing the devastating metabolism suffered by other conventional oral routes and leading to enhanced bioavailability [10]. For drugs with short half-life, extending their release would reduce frequent dosing and in our case would prevent early morning awakening without middle of night dosing.

Two main techniques were generally adopted for the preparation of buccoadhesive patches either hot melt extrusion or solvent casting. The latter was generally preferred as it offers low cost in addition to ease of processing [11]. Emulsification/casting/solvent evaporation technique is a modification of solvent casting technique for preparation of patches using polymers of opposing solubilities with the ultimate aim of controlling the release [12]. Further control can be applied by incorporating the drug into melt granules using melt-granulation technique prior to its inclusion into the patches. This approach might take buccal delivery to a whole new level in the field of controlled delivery.

Melting method includes melting of a physical mixture of the drug and the carrier, followed by sudden cooling of the molten mass over ice bath with continuous stirring, and then the final solidified mass is
pulverized and sieved. Melting method is also called melt-granulation technique [13,14]. Precirol is a mixture of 40% tri-, 45% di- and 14% monoglycerides of palmitic and stearic acids. It provides a matrix to sustain drug release and taste masking which make it ideal retardant to control drug release [15,16].

The aim of this study was to formulate ZLP into a buccoadhesive patch, in order to avoid the extensive first pass effect and improve its bioavailability. Concerning the control of ZLP release, we prepared Precirol-based ZLP melt-granules prior to its inclusion into the patch prepared with polymers of opposing solubilities. A Box-Behnken design was adopted to study the effect of formulation variables and to reach an optimized formula with the ultimate aim of preventing early morning awakening without middle of the night dose administration.

2. Materials and methods

2.1. Materials

Zaleplon (ZLP) was received as a kind gift from October Pharma, Egypt. Poly vinyl alcohol (PVA), Mwt 13,000–23,000; triethyl citrate (TEC); were purchased from Acros organics, Belgium. Precirol ATO 5 (Glyceryl distearate) was purchased from Gattefosse Co., St-Priest, France. Hydroxypropyl Methylcellulose K4M (HPMC), was purchased from Colorcon, Midland, USA. Ethyl cellulose (EC), viscosity 300 cps, 49% ethoxy; Dibutyl phthalate (DBP); and mucin from porcine stomach; were purchased from Sigma-Aldrich Co., St. Louis, USA. Eudragit® RS 100; was purchased from Degussa, Rohm GmbH and Co. KG, pharma polymer, Germany. All other reagents and chemicals used were of analytical reagent grade.

2.2. Preparation of ZLP Precirol-based melt-granules

Precirol was melted in porcelain evaporating dish over a hot plate at 90 °C. ZLP was added with continuous stirring for 15 min to get a homogeneous dispersion. Then, the molten mass was rapidly cooled down over an ice bath and allowed to solidify. Subsequently, the solidified mass was ground and pulverized in a glass mortar. The solid was sieved through 0.5 μm sieve [17,18].

2.3. Preparation of buccoadhesive patches of ZLP Precirol-based melt-granules by emulsification/casting/solvent evaporation process [11,12]

An aqueous solution of PVA (30%w/v) and a hydro-alcoholic solution (3:2) of HPMC (2%w/v) were prepared and plasticized with propylene glycol (20%w/v) of total polymeric content. Also an ethanolic solution of ethyl cellulose (5–10%w/v) was prepared and plasticized with dibutyl phthalate (50%w/v) of the polymer content. Then a predetermined weights of each solution was added along with a weight of ZLP Precirol-based melt-granules equivalent to 5 mg of ZLP.

The whole mixture was homogenized at 9500 rpm for 5 min using a homogenizer (Wisemix HG-15D Daihan scientific, Korea) at 25 °C to obtain finely divided melt-granules dispersed in an o/w emulsion. The o/w emulsion was maintained under stirring by a vortex mixer, 50 vibrations/min, at 25 °C for 2 min. The solution was then casted into cylindrical molds (diameter: 1.3 cm, thickness: 0.3 cm). A weight of the solution, containing melt-granules equivalent to 5 mg ZLP, was casted into each well. The patches were left to dry in air under an inverted funnel to prevent sudden evaporation.

The patches were dip-coated as an extra barrier for further control of the release. This was done by dipping the dried patches in the prepared coating solution of Eudragit RS 100 in acetone (10 %w/w) -plasticized with TEC (30%w/w) based on polymeric content-for 5 min. The resulting patches were air-dried, then stored at ambient temperature and humidity till use.

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<table>
<thead>
<tr>
<th>Table 1</th>
<th>Levels of independent variables investigated in the Box-Behnken design and constraints for optimization.</th>
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<tbody>
<tr>
<td>Variables</td>
<td>A: Precirol/Zaleplon ratio in melt-granules</td>
</tr>
<tr>
<td>FM 1</td>
<td>5: 1</td>
</tr>
<tr>
<td>FM 2</td>
<td>3: 1</td>
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<tr>
<td>FM 3</td>
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<td>FM 4</td>
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<td>FM 14</td>
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<tr>
<td>FM 15</td>
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<table>
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<tr>
<th>Responses</th>
<th>Constraints</th>
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<tr>
<td>Y1 = % Drug released after 3 h</td>
<td>minimize</td>
</tr>
<tr>
<td>Y2 = % Drug released after 12 h</td>
<td>maximize</td>
</tr>
<tr>
<td>Y3 = Mucoadhesion strength</td>
<td>maximize</td>
</tr>
</tbody>
</table>

2.4. Experimental design

For the preparation of ZLP buccoadhesive patches a three factor, three level Box-Behnken design was adopted using the Design Expert® software (Version 7, Stat-Ease Inc., Minneapolis, MN). Three factors namely, A: Precirol: ZLP ratio, B: HPMC solution: PVA solution ratio and C: Ethyl cellulose % were investigated as independent variables. The levels for these three parameters are shown in Table 1. According to the followed Box-Behnken design, 15 runs were tried. The preparation and release studies of the suggested formulae were done in random order. The 15 runs listed in standard order are shown in Table 2.

The response surface methodology (RSM) and multiple response optimization utilizing the fitted polynomial equations were used to search for the optimal formulation with a specific release rate at different time intervals and mucoadhesion strength. The drug release percentages at 3 h (Y1), 12 h (Y2) and the mucoadhesion strength (Y3) were the target responses. These dependent variables were chosen as shown Table 1.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Composition of different buccal patches according to Box-Behnken design.</th>
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</thead>
<tbody>
<tr>
<td>Number of runs</td>
<td>Precirol: Drug</td>
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<tr>
<td>FM 1</td>
<td>5: 1</td>
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<tr>
<td>FM 2</td>
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<td>FM 15</td>
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</table>

a A weight equivalent to 5 mg of ZLP is added in each individual patch.
| b 2% HPMC solution: 30% PVA solution (both are plasticized by propylene glycol 20% w/w of solid content), 0.66 g of the solution mixture was added in each individual patch.
| c The solution is plasticized by dibutyl phthalate 50% w/w of the solid content, 0.33 g of the solution was added in each individual patch.
(230.2 nm). The experiments were done in triplicates and the mean drug content ± SD was reported [19].

2.5.2. Thickness uniformity
The thickness of each patch was measured using micrometer at five different positions of the patch and the average thickness was calculated [19].

2.5.3. Percentage moisture loss (%ML)
Percentage moisture loss was carried out to check the integrity of patches at dry condition. Patches were weighed accurately and kept in a desiccator containing anhydrous calcium chloride. After 72 h the patches were removed and reweighed. The moisture loss (%) was calculated using the following formula [20]:

\[
\text{Moisture loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]

The experiment was carried out in triplicate and expressed as mean ± SD.

2.5.4. Water sorption (moisture uptake)
Patches were weighed accurately then were maintained at 75% relative humidity, this was achieved by keeping them in a desiccator containing saturated sodium chloride at 25 °C [21]. After 72 h the patches were removed and reweighed. The moisture uptake (%) was calculated using the following formula [20]:

\[
\text{Moisture uptake (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

The experiment was carried out in triplicate and expressed as mean ± SD.

2.5.5. Surface pH
The surface pH of the patch was determined by allowing it to swell in contact with 5 ml phosphate buffer saline (pH 6.8) for 2 h at room temperature then the electrode of the pH meter (Schott-Geräte GmbH, Germany) was brought in contact with patch surface and left to equilibrate for 1 min [22]. The surface pH was determined in triplicate and expressed as mean ± SD.

2.5.6. Mucoadhesion strength (Mucus glycoprotein assay)
The prepared patches were assessed regarding their mucoadhesion strength using mucus glycoprotein assay. The concept is to determine the amount of mucin adsorbed to the total surface area of the patch which is used to reflect the mucoadhesion strength. A standard mucin solution (1 mg/2 ml) is prepared in which each formula was incubated which is used to re\text{fl}ect the mucoadhesion strength. A standard mucin solution was used to stain and determine the free mucin so that the amount of adsorbed mucin can be calculated.

2.5.7. In-vitro drug release
The release of ZLP from the prepared patches was performed for 12 h at a temperature of 37 ± 0.5 °C using the USP Dissolution tester, rotating paddle (Apparatus II). The backed side of the patch was attached with cyanoacrylate to the side of rotating paddle. After 2 min, the vessel was filled with 250 ml phosphate buffer saline (pH 6.8) and stirred at a rotation speed of 50 rpm. Aliquots each of 3 ml from the dissolution medium were withdrawn at 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 12 h time intervals [24,25] and an equal volume of the fresh dissolution medium was added every time to maintain sink condition. The amount of ZLP released was measured spectrophotometrically at 230.2 nm. All release studies were done in triplicates.

2.5.8. Differential scanning calorimetry (DSC) studies
Differential scanning calorimetry was performed using Shimadzu differential scanning calorimeter, DSC-50. Samples were placed in flat bottomed aluminum pan and heated at a constant rate of 10 °C/minute, in an atmosphere of nitrogen in a temperature range of 20–350 °C. The DSC studies were performed for the pure drug, excipients and selected formula FM5.

3. Results and discussion

3.1. Patches characterization
Concerning weight uniformity, the prepared patches were found to vary from 101.9% ± 3.28%–109.69% ± 0.233%. The achieved results indicated that casting the drug in individual wells adjusted by weight rather than large sheets to be further cut into patches of definite area containing the required dose could ensure content uniformity [26]. As for the thickness of the prepared patches, it ranged from 0.522 ± 0.013 mm to 0.796 ± 0.018 mm, with small S.D. for all formulae indicating thickness uniformity.

Although it is highly recommended that the patches exhibit low moisture content to avoid microbial contamination, they should maintain low moisture level to avoid dryness and brittleness [20]. The %ML of the patches formulae ranged from 4.497% ± 0.118%–8.6% ± 0.09%. Water sorption of the patches formulae ranged from 0.132% ± 0.007%–3.586% ± 0.094%. The results of weight uniformity, thickness uniformity, moisture loss and water sorption are represented in Table 3. The measured pH of all prepared patches was found around 6.8 ± 0.21, which indicated their suitability for application into buccal mucosa without any irritation [27].

3.2. Statistical analysis of the Box-Behnken design and its interpretation

Results of the responses Y1 = Q3hr, Y2 = Q12hr and Y3 = mucoadhesion strength were statistically analyzed using ANOVA. ANOVA tests showed that the linear regression model was significant and fitting for mucoadhesion strength while the quadratic regression model was significant and fitting for Q3hr and Q12hr. The reduced equations, after omitting the non-significant model terms, are showed in Table 4.

3.2.1. Mucoadhesion strength
Taking into account that mucoadhesion is a sort of adsorption which is a surface phenomenon, therefore the amount of adsorbed mucin per total surface area of the patch was used to express the mucoadhesion strength of the formulae. The amount of adsorbed mucin was calculated by subtracting the free unadsorbed mucin from the total mucin. The mean results are represented in Table 3.

HPMC solution/PVA solution weight ratio was the only significant factor affecting mucoadhesion strength of the prepared patches formulae (P < 0.0001). Increasing the ratio of HPMC/PVA from 1:1 to 3:1 showed a significant increase in mucoadhesion strength of the prepared ZLP buccal patches formulae. HPMC is a non-ionic long-chained mucoadhesive polymer with many hydroxyl groups [28]. The mucoadhesion is due to formation of hydrogen bonds between these groups and the mucus components. By increasing HPMC proportion in the patch, more hydrogen bond are formed increasing the availability of binding sites, leading to substantial increase in mucoadhesion strength [28]. Fig. 1 illustrates the response surface plot for the effect of HPMC/ PVA on the mucoadhesion strength.

3.2.2. In-vitro drug release
Figs. 2–4 showed the in-vitro release profiles of ZLP from the prepared buccal patches formulae with the immediate-release market product, Siesta® and drug powder as illustrated in all the release figures. Q3hr, Q12hr were chosen to compare the in-vitro release profiles of ZLP from the prepared patches formulae.
ANOVA results revealed the negative effects of the Precirol/ZLP ratio (A) and percentage ethyl cellulose (C) \((p < 0.0001)\) and the positive effects of the PVA solution/HPMC solution weight ratio (B) \((p < 0.0001)\) on both Q3hr and Q12hr (see Figs. 5 and 6).

Regarding Precirol/ZLP ratio, increasing the ratio from 1:1 to 5:1 led to a decrease in drug release, this could be attributed to the lipophilicity of precirol. ZLP-Precirol melted granules added a constraint on the release of ZLP from the patch, the drug particles were included in the lipophilic Precirol which decreased the penetration of the dissolution medium to the drug, leading to a decreased dissolution, therefore by increasing the Precirol/ZLP ratio, a more retarded release was expected [29,30].

Regarding to HPMC/PVA ratio, increasing the ratio from 1:1 to 3:1 led to an increase in drug release. This could be explained by the high initial PVA concentration (30%w/w) and the low initial HPMC concentration (2%w/w), in case of the high HPMC solution/PVA (3:1), the total polymeric content in the patch was reduced. While in case of the low HPMC solution/PVA (1:1), the total polymeric content in the patch was increased, so by increasing the PVA solution content on the expense of the HPMC solution content (HPMC/PVA 1:1), more polymer was ultimately included in the patch, leading to increased swelling of patches in the dissolution medium was expected. As a result the diffusional path length of ZLP was increased and the ability of water to diffuse through the gel formed was decreased, resulting in a slower dissolution [31,32]. In addition, the high hydrophilic nature of HPMC resulted in its rapid dissolution and thus introducing pores into the patch matrix that would allow rapid diffusion of the dissolution medium into the patch and thus rapid drug dissolution from patches with higher HPMC solution/PVA solution ratio [32].

As for the concentration ethyl cellulose, increasing the concentration from 5% to 10% led to decrease in drug release, owing to its hydrophobic nature imparting another barrier to drug release in addition to that of Precirol and gel barrier effect of PVA. Ethyl cellulose acted as a shield that decreased the water penetration into the patch which affected pores formation and dissolution of ZLP to be leached from the film, therefore increasing percentage ethyl cellulose would prohibit water penetration, ZLP dissolution and slower release could be expected this is confirmed by previous findings where Kohda et al. and Marucci et al. concluded that ethyl cellulose decreased the release of lidocaine HCl and metoprolol succinate respectively [33,34].
3.3. Formulation optimization

After applying constraints on the design's variables, the Design Expert® software has chosen formula FM5 as the best achieved formula with desirability = 0.666. This patch showed optimum mucoadhesion strength (1507.12 ± 62.238 mcg/cm²) and controlled release profile (Q₃hr = 29.06% ± 0.73% and Q₁₂hr = 87.24% ± 1.71%).

3.4. Differential scanning calorimetry (DSC) studies

The DSC thermograms of zaleplon, different excipients used in the preparation of the buccal patches and the best achieved formula FM5 which showed optimum mucoadhesion strength (1507.12 ± 62.238 mcg/cm²) and controlled release profile (Q₃hr = 29.06% ± 0.73% and Q₁₂hr = 87.24% ± 1.71%) are illustrated in Fig. 7. The DSC

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Fig. 2. In-vitro release profiles of ZLP from the prepared buccal patches formulae FM (1–5), drug powder and Siesta® in phosphate buffer saline (pH 6.8) (mean ± SD).

Fig. 3. In-vitro release profiles of ZLP from the prepared buccal patches formulae FM (6–10), drug powder and Siesta® in phosphate buffer saline (pH 6.8) (mean ± SD).

Fig. 4. In-vitro release profiles of ZLP from the prepared buccal patches formulae FM (11–15), drug powder and Siesta® in phosphate buffer saline (pH 6.8) (mean ± SD).
thermograms of zaleplon alone showed endothermic peak at 187.74 °C, corresponding its melting point indicating its crystallinity. On the other hand all excipients didn't show any significant peaks except for precirol which showed a relatively wide endothermic peak that ranges from 55 to 57 °C [14,35]. It should be noticed that the peak of zaleplon has been completely disappeared in the thermogram of the selected formula FM5, this could possibly indicate successful incorporation of zaleplon into precirol melted granules in the prepared patch.

4. Conclusion

ZLP-Precirol melt granules in buccoadhesive patches were successfully prepared using melt granulation followed by emulsification/casting/solvent evaporation techniques. The patches showed uniform drug content and thickness indicating their homogeneity, also they showed minimum water sorption and moisture loss which indicate their ability to minimize microbial contamination and prevent brittleness. The patches showed acceptable pH range which is expected to avoid any irritation to buccal mucosa. Furthermore formula FM5 (composed of Precirol/ZLP ratio of 5:1, HPMC solution/PVA solution weight ratio of 3:1, and 7.5% Ethyl cellulose) showed optimum mucoadhesion strength (1507.12 ± 62.238 mcg/cm²) and controlled release profile (Q3hr = 29.06% ± 0.73% and Q12hr = 87.24% ± 1.71%) compared to Siesta® marketed product which could be a reliable alternative for drugs suffering from extensive first pass hepatic metabolism along with short half-life. Concerning ZLP this approach could be promising to prevent early morning awakening without middle of night dose administration.

Conflicts of interest

The authors have no conflict of interest to declare.
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References