FORMULATION, OPTIMIZATION AND IN VITRO EVALUATION OF RAPID DISINTEGRATING AND MUCOADHESIVE SUBLINGUAL TABLETS OF LORAZEPAM

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Abstract

Lorazepam a benzodiazepine drug is used as an antianxiety, sedative, hypnotic, and anticonvulsant drug. Bypassing the enterohepatic recirculation and first-pass metabolism of lorazepam by sublingual delivery of this drug may accelerate the onset of its action. The objective of this study was the formulation, optimization and in vitro characterization of mucoadhesive sublingual tablets of lorazepam. To optimize the formulation of the mucoadhesive sublingual tablets of lorazepam seven variables including: disintegrating agent type (Primojel or Kollidone), filler [Silicified microcrystalline cellulose (SMCC) or Avicel PH 101], carrier powder (mannitol or lactose), disintegrating agent content (5 or 10%), lubricant type (Mg-stearate or Aerosil), drug content (1 or 2 mg) and mixing time (12 or 24 h) were studied by the Taghuchi design and nine different formulations were prepared. The tablets were tested for their thickness, hardness, weight variation, drug content uniformity, assay, porosity, disintegration time, bioadhesivien tensile strength and dissolution efficiency and the effect of different studied formulation parameters were studied on their properties. The formulation of tablets was optimized considering their dissolution efficiency within 10 minutes (DE_{10}). The optimum formulation obtained from 2 mg of the drug, 1.2 mg Mg-stearate, 6 mg of Primojel, 20.8 mg of Avicel, 90 mg of mannitol which was mixed for 12 hours with the drug. This tablet with the DE_{10} of 81.30±2.50%, bioadhesion of 34.15±0.15%, and disintegration time of about 14 sec seems promising as a rapid disintegrating and mucoadhesive sublingual tablet for lorazepam.

Rezumat

Lorazepam este un medicament benzodiazepinic utilizat ca antianxios, sedativ, hipnotic și anticonvulsivant. Ocolind bariera enterohepatică și efectul primului pasaj, metabolizarea lorazepamului prin administrare sublinguală a acestui medicament poate accelera debutul acțiunii sale. Obiectivul acestui studiu a fost formularea, optimizarea și caracterizarea in vitro a comprimatelor mucoadexe sublinguale de lorazepam. Pentru a optimiza formularea comprimatelor sublinguale mucoadexe au fost studiate prin proiectarea Taghuchi șapte variabile, inclusiv: dezintegrant (Primojel sau Kollidone), agent de umplutură (Celuloză microcristalină silicifiată (SMCC) sau Avicel PH 101), pulbere purtătoare (mannitol sau lactoză), cantitatea de substanță activă (1 sau 2 mg) și timpul de amestecare (12 sau 24 ore) - s-au utilizat noau formulări diferite. Au fost testate următorii parametri de control ai comprimatelor: grosimea, duritatea, uniformitatea masei, uniformitatea cantității în substanță activă, cantitatea de substanță activă, porozitatea, timpul de dezintegrare, rezistența bioadheziunii la tracțiune și eficiența dizolvării. Au fost studiate influențele diferențelor parametri de formulare studiați asupra proprietăților comprimatelor. Formularea comprimatelor a fost optimizată luându-se în considerare eficiența lor la dizolvarea în 10 minute (DE_{10}). Formularea optimă a fost obținută din 2 mg de substanță activă, amestecată timp de 12 ore cu 1,2 mg stearat de magneziu, 6 mg Primojel, 20,8 mg de Avicel și 90 mg de manitol. Acest comprimat cu DE_{10} de 81,30±2,50%, bioadheziea de 34,15±0,15%, iar timpul de dezintegrare de circa 14 sec pare o formulare promițătoare cu o dezintegrare rapidă pentru un comprimat sublingual mucoadexiv cu lorazepam.

Keywords: Lorazepam, rapid disintegrating tablets, mucoadhesive, sublingual tablets

Introduction

Lorazepam,7-chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-1,4-benzodiazepine-2-one, with the chemical formula \( C_{13}H_{12}ClN_{2}O_{2} \) and the molecular weight of 321.2 g, is a white or almost white polymorphic crystalline powder; practically insoluble in water (80 μg/mL) and \( P = 2.4 \). It is a short acting benzodiazepine clinically used as an antianxiety, sedative and hypnotic, and anticonvulsant drug in the treatment of anxiety disorders, insomnia and status epilepticus, respectively. It is administered orally, parenterally, or sublingually [1, 2]. After oral administration, lorazepam is absorbed readily from the gastrointestinal tract and reaches to its peak plasma concentration after about 2 hours. Its bioavailability and protein binding is approximately 90% and 85%, respectively.
Lorazepam undergoes hepatic metabolism and is inactivated through glucuronidation. Its elimination half-life is between 10 to 20 hours and its plasma clearance is about 1 mL/min/kg [1, 2]. Sometimes its sublingual tablets are used in similar doses as the conventional oral tablets. Lorazepam sublingual tablets are considered preferable as premedication, anticonvulsant, and anxiolytic, in the case of cataract surgery, outpatient minor oral surgery, colonoscopy, and nervous dental patients, as well as childhood serial seizures [2-9].

Regarding its high partition coefficient (log P), lorazepam demonstrates a high rate of absorption allowing sublingual administration [1, 10]. After 30-40 min from sublingual administration, it reaches a higher plasma concentration in comparison with the intramuscular administration; however, the intramuscular route results in a higher level after 1 hour [10].

When achieving rapid onset of action is desired, for instance, in the treatment of acute disorders, parenteral administration could be considered as an appropriate choice. However, it is not always a preferable route of administration by the patient; hence, a growing effort is dedicated to non-parenteral dosage forms that provide immediate absorption. Among these dosage forms, tablets are considered as the most convenient one, regarding either the production procedure, or the usage by the patient. Nevertheless, as a consequence of their highly variable gastric retention time in oral administration, the desirable rapid onset of action could not be achieved unless an oromucosal drug delivery system is developed. Therefore, the sublingual mucosa would be a suitable absorption site for the tablet, resulting in a convenient dosage form which is readily absorbed and increases the bioavailability since it takes the advantage of highly vascular sublingual mucosa to enter the systemic circulation directly and to bypass the first pass metabolism of the drug in the liver.

The sufficient solubility and stability, short disintegration time, high permeation through mucosal layer, and fast dissolution rate as well as enough contact time at the site of administration are the demanding characteristics for the active pharmaceutical ingredients and other formulation excipients used in the formulation of this type of tablets. Otherwise, the bioavailability would adversely not reach the desired level since a portion of the drug would be swallowed instead of being absorbed from the area. To minimize the probability of swallowing the active pharmaceutical ingredient (API) before completing its absorption in the sublingual area, one approach is to integrate mucoadhesive additives into the formulation. This could help to increase the duration of retaining the tablet inside the sublingual area [11]. Considering the enterohepatic recirculation and first-pass metabolism of lorazepam [12], mucoadhesive sublingual tablets would be a rational solution to bypass its metabolism and accelerate the onset of its action. This new sublingual tablet formulation may also hold potential for substances where a rapid onset of effect is desirable. These tablets are a novel drug delivery system for rapid absorption of lorazepam through which are disintegrated rapidly and produce mucoadhesive particles in the sublingual area. The aim of the present study was the formulation, optimization and in vitro evaluation of the mucoadhesive sublingual tablets of lorazepam.

Materials and Methods

Materials

Lorazepam (Profarmaco, Italy), Avicel PH101 (FMC, US), Silicified microcrystalline cellulose (SMCC or Prosolv 50, FMC, US), Primojel and Aerosil (JRS, Germany), Kollidone (BASF, Germany), Mg-stearate (LERAT, Germany), lactose (MEGGLL, Germany), mannitol (Merck, Germany), Croscarmellose Sodium or Ac-Di-Sol® (FMC BioPolymer, US) and Mucin (Sigma, US).

Preparation of mucoadhesive sublingual tablets

A pilot study was applied to define duration of mixing time and the disintegrant type. The tablets were prepared using a single punch tabletting machine (GmbH-KS Killian, Germany). In the preliminary studies short times of mixing (10 min, one hour and 6 hours) were checked and they didn’t homogeneity results when testing by content uniformity test. For this reason longer times were studied. In formulations of PB1 and PB2 (Table 1) the drug was mixed with the carrier powder using a mixer (RAR50, Heidolph, Germany) for 24 and 48 hours, respectively. Various tests such as weight variation, content uniformity, hardness, assay and thickness were performed on them. Since formulation PB2 could not pass the content uniformity test, the duration of mixing was adjusted to 12 and 24 hours. In order to select the type of disintegrating agent Kollidone, Croscarmellose, hydroxyl propyl cellulose and Primojel were tested. The same tests were done on three formulations, B1, PB3 and PB4 as mentioned above. Since the formulations PB1 and B1 had the shortest disintegration time, Kollidone and Primojel were selected as disintegrating agents.

Regarding the preformulation studies, seven variables each in two levels were selected to be studied by the Taghuchi design to optimize the formulation of the tablets of lorazepam (Table II). Nine different formulations (B1-B9) (Table I) were suggested by Qualitech 4 software (Nutek, Inc., US). All the formulations mentioned in Table II contained constant amounts of 1% of lubricant, 90...
mg of carrier powder and other ingredients. The total tablets weight was adjusted to 120 mg. Initially the carrier of the drug was mixed with the API for 12 or 24 hours in a tumbler mixer, and then the disintegrating agent was added and mixed for an extra 30 minutes. Finally the lubricant was added and mixed for further two minutes. The resulted powder was pressed to tablet (Figure 1).

![Schematic representation of different steps in the production of mucoadhesive sublingual tablets of lorazepam](image)

**Figure 1.**

Schematic representation of different steps in the production of mucoadhesive sublingual tablets of lorazepam

### Table I

| Ingredients of different formulations of mucoadhesive sublingual tablets of lorazepam |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Drug (mg) | Mannitol (mg) | Lactose (mg) | SMCC (mg) | Avicel (mg) | Primojel (mg) | Kollidon e (mg) | NaCMC (mg) | HPC (mg) | Mg-stearate (mg) | Aerosil (mg) |
| PB1    | 2 | 90 | 6 | 20.8 | 90 | 2 |
| PB2    | 48 | 1.2 | 6 | 20.8 | 90 | 2 |
| PB3    | 24 | 1.2 | 6 | 20.8 | 90 | 2 |
| PB4    | 24 | 6 | 20.8 | 90 | 2 |
| B1     | 24 | 1.2 | 6 | 20.8 | 90 | 2 |
| B2     | 12 | 1.2 | 12 | 15.8 | 90 | 1 |
| B3     | 12 | 1.2 | 6 | 21.8 | 90 | 1 |
| B4     | 24 | 1.2 | 6 | 20.8 | 90 | 2 |
| B5     | 12 | 1.2 | 6 | 20.8 | 90 | 2 |
| B6     | 24 | 1.2 | 12 | 15.8 | 90 | 1 |
| B7     | 24 | 1.2 | 6 | 21.8 | 90 | 1 |
| B8     | 12 | 1.2 | 12 | 14.8 | 90 | 2 |
| B9*    | 12 | 1.2 | 6 | 20.8 | 90 | 2 |

*The optimum formulation*

### Table II

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disintegrating agent type</td>
<td>Primojel</td>
<td>Kollidon e</td>
</tr>
<tr>
<td>Filler</td>
<td>SMCC</td>
<td>Avicel PH 101</td>
</tr>
<tr>
<td>Carrier</td>
<td>Mannitol</td>
<td>Lactose</td>
</tr>
<tr>
<td>Disintegrating agent content (%)</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Lubricant type</td>
<td>Mg-stearate</td>
<td>Aerosil</td>
</tr>
<tr>
<td>Drug content (mg)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Mixing time (h)</td>
<td>24</td>
<td>12</td>
</tr>
</tbody>
</table>

**Weight variation of tablets**

Twenty tablets of each batch were weighed and their mean, standard deviation and CV% was calculated according to USP standards. For tablets weighing less than 130 mg, 10% standard deviation would be acceptable.

**Hardness**

The hardness of 10 tablets of each formulation was measured using a tablet hardness tester (Erweka, GmbH, Germany).

**Assay**

Twenty tablets of each batch, equal to 5 mg of lorazepam, were ground into powder and added to 40 mL of ethanol. After being shaken for 1 hour, the solution was diluted to 50 mL and 5 mL of this solution was diluted with ethanol to 100 mL. The absorbance was measured using a UV-Vis spectrophotometer (UVmini-1240CE-Shimadzu,
Japan) at $\lambda_{\text{max}} = 230$ nm. The procedure was performed in triplicate.

**Content uniformity**

This test was performed on 10 tablets separately so that, one tablet was ground into powder and added to 40 mL of ethanol. The obtained solution was diluted to 50 mL after shaking for one hour. Then, it was centrifuged and 1 mL of the supernatant was diluted to 10 mL and its absorbance was measured spectrophotometrically at 230 nm.

**Thickness of tablets**

The thicknesses of ten tablets of each batch were measured by a micrometer calliper.

**Tensile strength of tablets**

The hardness of tablets of each batch was measured as mentioned before (hardness) and the resulted data were put into the following equation in which $\delta_d$ represents the tensile strength, $F_d$ is the hardness in N, D is the diameter of tablets in mm, and H represents the height of the tablets in mm.

$$\delta_d = \frac{2F_d}{\pi DH} \quad \text{Eq. 1.}$$

**Disintegration time of tablets**

The disintegration time of 6 tablets of each batch was determined using a dissolution test instrument (PTWS3, Pharma-test, Germany).

**Dissolution test**

The dissolution medium was 500 mL of phosphate buffer (pH 6.8) and dissolution instrument II of USP i.e., rotating paddle was used at 50 rpm and 37.5 ± 0.5°C. Five ml samples were taken at 1, 3, 5, 7, and 10 minutes and replaced by 5 mL of phosphate buffer. After centrifugation of samples the absorbance was measured at 230 nm. Finally, the cumulative percent of the dissolved drug was plotted versus time. This test was performed on 3 tablets of each batch. Then the percentage of dissolution efficiency after 10 minutes (DE$_{10}$%) was calculated by the following equation:

$$\text{DE}_{10} = \left( \frac{\int_0^t y \, dt}{Y_{100} \times t} \right) \times 100 \quad \text{Eq. 2},$$

where $y$ is the released drug in each sampling time, $Y_{100}$ is the 100 percent of drug released in time t.

The resulted response was analysed by Qualitech 4 software to optimize the studied formulations of the tablets.

**Tablet porosity**

Tablets of each batch were ground into powder to obtain at least 2 g of powder and the real density of the resulted powder was measured using a helium pycnometer (Quantachrome, US). The apparent volume of the tablets was calculated through the following equation assuming that the tablets are in a cylindrical shape.

$$V = \frac{1}{3} \pi r^2 h \quad \text{Eq. 3.}$$

Then, the apparent density was measured using the apparent volume of the tablets. Eq. 4 was used to calculate tablets porosity in which $\varepsilon$ represents the porosity of the tablets, $\rho_r$ is the apparent density, and $\rho_t$ is the real density of the tablets.

$$\varepsilon = \left( 1 - \frac{\rho_r}{\rho_t} \right) \times 100 \quad \text{Eq. 4.}$$

**Mucoadhesive properties of the tablets**

The mucoadhesive property of the tablets was evaluated by the modified falling liquid film method. For this test bovine sublingual tissue was frozen during the first hour and artificial saliva (containing: NaHCO$_3$ 0.21 g, NaCl 0.43 g, KCl 0.75 g, CaCl$_2$·2H$_2$O 0.91 g, mucin 2.7 g, distilled water qs to 1 L) was produced [13].

The sublingual tissue was cut into 3×5 cm pieces and placed on a ramp surface. 120 mg of the
powdered tablets was placed at the distance of 1 cm from the upper part of the tissue and was washed for 5 min with artificial saliva at 37°C with the flow rate of 2 mL/min (Figure 2). Afterwards, the artificial saliva solution was gathered, dried and weighed.

Statistical Analysis
Optimization of the tablets formulation was done by QualiTech 4 software. One way analysis of variance (ANOVA) along with Dunnan’s post hoc test was applied for comparing different responses by SPSS11 software.

Results and Discussion

Weight variation
The weights of all tablets were in the range of acceptable variations. According to the USP standards for tablets weighing less than 130 mg, the acceptable range is 10% of mean weight and none of the formulations were out of this limit. As the weight of the tablets was adjusted to 120 mg the acceptable upper limit was 132 mg and the lower limit was 108 mg. Although the ratio of API to excipient was low in the tablets, the content uniformity was acceptable and the optimal formulation had a suitable dissolution, probably due to the application of ordered mixtures. The major part of ordered mixtures is constructed by the carrier; therefore, it should possess high compressibility so mannitol and lactose were included in the formulation. SMCC and Avicel PH101 were used as filler to balance the formulations.

Hardness
Mean and standard deviation of the hardness of tablets is reported in Table III. All tablets had hardness values above 40 N.

Table III
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Tensile strength ± SD (N/mm²)</th>
<th>Drug content ± SD (mg)</th>
<th>Hardness ± SD (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>2.02 ± 0.05</td>
<td>1.98 ± 0.10</td>
<td>64.00 ± 2.80</td>
</tr>
<tr>
<td>B2</td>
<td>1.39 ± 0.29</td>
<td>0.98 ± 0.07</td>
<td>45.33 ± 8.38</td>
</tr>
<tr>
<td>B3</td>
<td>1.34 ± 0.16</td>
<td>1.01 ± 0.06</td>
<td>42.66 ± 2.51</td>
</tr>
<tr>
<td>B4</td>
<td>1.55 ± 0.16</td>
<td>2.03 ± 0.08</td>
<td>49.01 ± 6.24</td>
</tr>
<tr>
<td>B5</td>
<td>1.40 ± 0.08</td>
<td>2.02 ± 0.07</td>
<td>45.33 ± 3.15</td>
</tr>
<tr>
<td>B6</td>
<td>1.52 ± 0.17</td>
<td>0.99 ± 0.07</td>
<td>49.66 ± 6.80</td>
</tr>
<tr>
<td>B7</td>
<td>1.16 ± 0.07</td>
<td>1.00 ± 0.03</td>
<td>55.00 ± 3.60</td>
</tr>
<tr>
<td>B8</td>
<td>1.39 ± 0.15</td>
<td>2.03 ± 0.07</td>
<td>45.33 ± 6.11</td>
</tr>
<tr>
<td>B9</td>
<td>1.34 ± 0.05</td>
<td>2.04 ± 0.06</td>
<td>42.66 ± 2.08</td>
</tr>
<tr>
<td>PB1</td>
<td>2.06 ± 0.05</td>
<td>2.06 ± 0.06</td>
<td>44.63 ± 3.12</td>
</tr>
<tr>
<td>PB2</td>
<td>2.21 ± 0.14</td>
<td>2.21 ± 0.14</td>
<td>51.11 ± 5.43</td>
</tr>
<tr>
<td>PB3</td>
<td>2.01 ± 0.07</td>
<td>2.01 ± 0.07</td>
<td>42.94 ± 6.19</td>
</tr>
<tr>
<td>PB4</td>
<td>2.04 ± 0.05</td>
<td>2.04 ± 0.05</td>
<td>45.24 ± 2.78</td>
</tr>
</tbody>
</table>

Content uniformity
The preliminary studies short times of mixing (10 min, one hour and 6 hours) didn’t show good homogeneity results and varied between 0.6 to 1.5 mg in 1 mg tablets. For this reason long mixing periods were included in the study as stipulated in Table I. The possibility of achieving an interactive mixture with high dose homogeneity containing an extremely low proportion of a micronized drug was also reported by Sundell-Bredenberg and Nyström [14] who prepared binary mixtures containing coarse particulate mannitol and sodium salicylate particles after 72 hours mixing. Bredenberg et al. [11] also reported 48 hours mixing at 90 rpm for fentanyl citrate mixing with mannitol in a tumbling mixer for producing the interactive mixture of this drug with the carrier.

In all studied formulations the drug content in 20 assayed tablets was in the range of the standards of USP and was between 90-115% of the claimed amount and the CV% was less 6%. The amount of API was in the range of 0.9 to 1.1 mg in 1 mg tablets, and between 1.8 and 2.2 mg in 2 mg tablets.

Tensile strength of tablets
Mean and standard deviation of the tensile strength of 3 tablets of each formulation is presented in Table III. The average tensile strength of tablets in studied formulations and the effect of different levels of the each variable on the tensile strength is plotted using the Qualitech 4 software in Figure 3. As Figure 4 shows, the most effective factor on increasing the tensile strength of tablets was the mixing time of the drug and the carrier powder with an effect of about 52% and the most tensile strength was obtained when this mixing time was increased from 12 to 24 hours (Figure 3). By reducing the mixing time, the mechanical bounds among carrier particles become weaker and consequently the disintegration time was reduced due to faster penetration of gastrointestinal liquids into the tablets (Figures 5 and 6) but this factor did not have any significant effect on dissolution efficiency.
(Figure 13) which may be due to the poor solubility of the drug. Fast disintegration could not affect the dissolution rate.

**Figure 3.**
The effect of different studied variables including:
- a) disintegrant type,
- b) filler type,
- c) carrier type,
- d) disintegrant content,
- e) lubricant type,
- f) drug content, and
- g) mixing time on the tensile strength of mucoadhesive sublingual tablets of lorazepam.

![Graph showing the effect of different variables on tensile strength](image)

**Figure 4.**
Contribution percent of the different studied variables including:
- a) disintegrant type,
- b) filler type,
- c) carrier type,
- d) disintegrant content,
- e) lubricant type,
- f) drug content, and
- g) mixing time on the tensile strength of mucoadhesive sublingual of lorazepam.

As it could be observed in the diagram, the duration of mixing of the drug and the carrier was the most effective parameter in increasing the tensile strength of the tablets. Carrier type was 13.26% effective on tensile strength of tablets (Figure 4) and the best results obtained when mannitol was used instead of lactose (Figure 3). According to Figure 4, the type of the disintegrating agent was only 9.96% effective on tensile strength of tablets. The tensile strength of tablets was reduced by increasing the amount of disintegrating agent from 5 to 10%, changing the SMCC to Avicel PH101, which has high compressibility, and using aerosol instead of Mg stearate.

**Disintegration time**
Mean and standard deviation of the disintegration times of 3 tablets of each batch are reported in Table IV. The average disintegration time of each formulation along with the effect of different levels of the studied variables on the average disintegration time of all formulations is plotted in Figure 5 using the Qualitech 4 software.
Table IV

Bioadhesion, disintegration time, porosity and dissolution efficiency of mucoadhesive sublingual tablets of lorazepam

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Bioadhesion ± SD (%)</th>
<th>Disintegration time ± SD (sec)</th>
<th>Porosity ± SD (%)</th>
<th>DE10 ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>36.85 ± 0.15</td>
<td>14.00 ± 3.00</td>
<td>44.30 ± 5.19</td>
<td>69.51 ± 3.02</td>
</tr>
<tr>
<td>B₂</td>
<td>89.35 ± 0.25</td>
<td>15.60 ± 2.08</td>
<td>43.30 ± 1.93</td>
<td>45.80 ± 3.73</td>
</tr>
<tr>
<td>B₃</td>
<td>52.85 ± 2.55</td>
<td>19.00 ± 1.73</td>
<td>44.66 ± 3.29</td>
<td>75.61 ± 1.63</td>
</tr>
<tr>
<td>B₄</td>
<td>35.35 ± 0.75</td>
<td>16.33 ± 3.05</td>
<td>26.33 ± 2.62</td>
<td>64.95 ± 3.53</td>
</tr>
<tr>
<td>B₅</td>
<td>36.70 ± 0.10</td>
<td>54.66 ± 4.50</td>
<td>27.36 ± 0.36</td>
<td>25.65 ± 3.61</td>
</tr>
<tr>
<td>B₆</td>
<td>56.15 ± 0.45</td>
<td>214.66 ± 23.70</td>
<td>39.66 ± 2.49</td>
<td>10.69 ± 3.56</td>
</tr>
<tr>
<td>B₇</td>
<td>39.05 ± 4.05</td>
<td>53.00 ± 2.64</td>
<td>22.00 ± 0.08</td>
<td>30.41 ± 1.19</td>
</tr>
<tr>
<td>B₈</td>
<td>45.95 ± 7.68</td>
<td>26.66 ± 3.78</td>
<td>34.66 ± 2.05</td>
<td>32.34 ± 2.62</td>
</tr>
<tr>
<td>B₉</td>
<td>34.15 ± 0.15</td>
<td>14.00 ± 1.00</td>
<td>37.33 ± 5.70</td>
<td>81.30 ± 2.50</td>
</tr>
<tr>
<td>PB₁</td>
<td></td>
<td>36.66 ± 4.01</td>
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<tr>
<td>PB₂</td>
<td>48.05 ± 5.01</td>
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</tr>
<tr>
<td>PB₃</td>
<td>264.33 ± 36.00</td>
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</tbody>
</table>

Figure 5.

The effect of different studied variables including:
- a) disintegrant type,
- b) filler type,
- c) carrier type,
- d) disintegrant content,
- e) lubricant type,
- f) drug content,
- g) mixing time on the disintegration of mucoadhesive sublingual tablets of lorazepam.

The bar graph of the contribution percent of the effects of different variables on the disintegration time of sublingual tablets of lorazepam is presented in Figure 6. As it could be observed in this diagram, the type of disintegrating agent was the most effective variable (with 35% effectiveness) on decreasing of the disintegration time of the tablets. Primojel resulted in faster disintegration comparing to Kollidon as shown in Figure 5. According to other studies [15] the appropriate amount of Primojel in rapid disintegrating tablets is 7%. In the present work, the percent of disintegrating agent was used in two levels of 5 and 10 percent to evaluate the effect of this factor on disintegration time of the tablets which was 8.85% effective on this response (Figure 6). As it can be observed in Figure 5 increasing the disintegrating agent from 5 to 10 percent reduced the disintegration time and enhanced the binding effect.
Figure 6 also indicates that the effect of carrier type on the disintegration time was 12.92% and mannitol was the best carrier due to its higher solubility compared to lactose [16]. As observed in Figure 5 the disintegration time reduced by changing the carrier powder from lactose to mannitol. Avicell PH101 was suitable filler in order to achieve the minimum disintegration time (Figure 5) and the effect of this factor on the disintegration time was 10.58% (Figure 6). This would be due to higher hydrophilicity of Avicell PH101 comparing to SMCC which is a mixture of colloidal silicon dioxide and microcrystalline cellulose [16]. The amount of API in the tablets was 11.45% effective on reducing the disintegration time (Figure 6); where, changing the amount of API from 1 to 2 mg resulted in an increment in the disintegration speed (Figure 5).

Figure 6 shows that the type of lubricant was 9.08% effective on reducing the disintegration time and the tablets containing Aerosil disintegrated faster and better than those containing magnesium stearate (Figure 5) because colloidal silicon dioxide absorbs water 9 fold higher than starch [17].

The mixing time of the drug and carrier was 10.32% effective on reducing the disintegration time (Figure 6) and the best results obtained when the mixing time was 12 hours, according to Figure 5.

Mucoadhesive properties of the tablets
Mean and standard deviation of the percentages of the powder remained on the sublingual tissue was measured after 5 min and the results are reported in Table IV. The average mucoadhesive properties of each formulation along with the effect of different levels of the studied variables on the bioadhesion of all formulations are plotted in Figure 7 using the Qualitech 4 software.

The bar graph of the contribution percent of the effects of different variables on the bioadhesion of the tablets of lorazepam is presented in Figure 8. As it could be observed in this diagram, the drug content, amount of the disintegrating agent, and the mixing time of the drug and the carrier powder were the most effective variables on increasing the mucoadhesiveness of tablets.

The most effective factor which increased bioadhesion of the tablets was the API dose with 37.23% effectivenesst (Figure 8). One mg increase in the amount of API reduced the bioadhesion of the tablets (Figure 7). This may be due to production of drug agglomerates during the mixing of the powders and changing the ordered mixing of the drug-carrier to a random mixture [18] which consequently reduces the coverage of the drug on the surface of the carrier powder and exposure of the water soluble carrier which in turn reduces the bioadhesion of the tablets.

Although the carrier type did not have a significant effect (p > 0.05) on the bioadhesion of the tablets (Figure 7); only 4.96% effectiveness, but the addition of a water soluble agent such as mannitol or lactose to the mucoadhesive polymer reduced its efficacy (Figure 8). This was possibly because less amount of water remained available for the polymer to swell and form hydrogen bonds [18]. Mannitol resulted in more bioadhesion than lactose (Figure 7). This is in accordance to the previous studies on buccal tablets of propranolol and the authors showed that in these tablets which contained mannitol or lactose as carrier and Kollidon as mucoadhesive agent, mannitol was more effective than lactose on bioadhesion of the tablets [19].

Increasing the amount of disintegrating agent was also effective on increasing bioadhesiveness of the tablets. This was 20.56% effective on this response (Figure 8), since the disintegrating agents were used as the mucoadhesive agent too [20-22].

Another effective factor on increasing the bioadhesiveness was mixing time of the drug and the carrier, which was 18.03% effective (Figure 8). The higher bioadhesion was achieved at 12 hours of mixing compared to the 24 hours (Figure 7). This could be described regarding the fact that more increment in the mixing time could result in agglomeration of the powder due to induced electrostatic charges.

The type of filler was also 11.48% effective on the bioadhesiveness (Figure 8), where the powders containing SMCC demonstrated a better bioadhesiveness in comparison to Avicel PH101 (Figure 7) which is due to higher hydrophilicity of Avicel.
The best disintegrating agent in increasing the bioadhesiveness was Promojel (Figure 7) with an effect of 7.29% (Figure 8).

**Figure 7.**
The effect of different studied variables including:
- a) disintegrant type,
- b) filler type,
- c) carrier type,
- d) disintegrant content,
- e) lubricant type,
- f) drug content,
- g) mixing time on the bioadhesion of mucoadhesive sublingual tablets of lorazepam.

**Figure 8.**
Contribution percent of the different studied variables including:
- a) disintegrant type,
- b) filler type,
- c) carrier type,
- d) disintegrant content,
- e) lubricant type,
- f) drug content,
- g) mixing time on the bioadhesion of mucoadhesive sublingual tablets of lorazepam.

**Porosity of tablets**
The average porosity of 17 formulations of tablets are summarized in Table IV. The effect of different levels of the studied variables on the porosity of all formulations is plotted in Figure 9 using the Qualitech 4 software.
Figure 9.
The effect of different studied variables including: a) disintegrant type, b) filler type, c) carrier type, d) disintegrant content, e) lubricant type, f) drug content, and g) mixing time on the porosity of mucoadhesive sublingual tablets of lorazepam.

The bar graph of the contribution percent of the effects of different variables on the porosity of the tablets of lorazepam is presented in Figure 10. As it could be observed in the diagram, the type of lubricant and, to some extent, the type of disintegrating agent were the most effective parameters on decreasing the porosity of the tablets.

According to the literature addition of Aerosil to tablets would increase diameter and volume of pores in the resulted tablets with a linear relationship to the amount of Aerosil [19]. However, in our present work, changing the lubricant had an effect of 38.38% on the tablets porosity (Figure 10) and replacing Magnesium stearate with Aerosil reduced the porosity (Figure 9).

Other parameters influencing the tablet porosity included the type of disintegrating agent which demonstrated the highest porosity when Kollidon was used (Figure 9) possible as Kollidon has more compressibility than Primojel. According to Figure 10 the filler type had an effect of 13.94% on increasing the tablets porosity and the most porous tablets were those containing Avicel PH101 (Figure 9) which could be due to higher inter-particular forces in SMCC [22]. However, the carrier type and the disintegrant content did not have significant effect on the porosity of the tablets (Figure 10).

Dissolution of tablets
The percentage of released drug from different formulations was plotted versus time as seen in Figure 11. The dissolution efficiency percent in 10 min (DE_{10%}) was calculated as summarized in...
Table IV. The effect of different levels of the studied variables on the DE$_{10}\%$ of all formulations is plotted in Figure 12 using the Qualitech 4 software.

**Figure 11.** Release profiles of lorazepam from different studied formulation of mucoadhesive sublingual tablets.

**Figure 12.** The effect of different studied variables including: a) disintegrant type, b) filler type, c) carrier type, d) disintegrant content, e) lubricant type, f) drug content, and g) mixing time on the DE$_{10}\%$ of mucoadhesive sublingual tablets of lorazepam.

The bar graph of the contribution percent of the effects of different variables on the DE$_{10}\%$ of the tablets of lorazepam is presented in Figure 13. As it can be observed in this diagram, the type of disintegrating agent was the most effective parameter in decreasing the DE$_{10}\%$ of the tablets and Primojel was the desirable disintegrating agent. Better performance of Primojel in comparison to Kollidon and starch 1500 was also reported in the previous researches on fast dissolving tablets of lorazepam.
acetaminophen [24] and in comparison to Crosscarmellose in hydrochlorothiazide pellets [25]. In contrast, the extent and rate of dissolution of fast dissolving tablets of terfenadine containing Kollidone was higher in comparison to Primojel [26]. These results may be due to acidic dissolution medium which has negative effects on the efficacy of Primojel as a fast disintegrating agent since Primojel has the best release profile in neutral pH [27]. Besides, in the mentioned study, gas producing agents were used to reduce the disintegration time which resulted in conversion of tablets containing Kollidone into smaller particles [24]. Figure 12 demonstrates that by changing the disintegrating agent from Kollidon to Primojel the increment of DE\textsubscript{10}% happened. Previous studies show that Primojel is a more suitable disintegrating agent for sublingual tablets which should be disintegrated at pH 6.8.

**Figure 12.**

Contribution percent of the different studied variables including:
- a) disintegrant type,
- b) filler type,
- c) carrier type,
- d) disintegrant content,
- e) lubricant type,
- f) drug content,
- g) mixing time on the DE\textsubscript{10}% of mucoadhesive sublingual tablets of lorazepam.

It is also concluded from this figure that changing the filler agent from SMCC to Avicel, had an increasing effect on the DE\textsubscript{10}% of lorazepam. This is because SMCC appears slightly more hydrophobic than Avicel PH101 due to the presence of colloidal silicon dioxide in its composition [28]. Figure 13 shows that increasing the percent of the disintegrating agent decreased the amount of DE\textsubscript{10}%. The effect of this factor on DE\textsubscript{10}% was 7.66% (Figure 13). Regarding that disintegrating agents could also act as binder in the formulation, overusing the disintegrating agents would cause reverse effects [29].

DE\textsubscript{10}% was independent to the type of the carrier and as observed in Figure 13, the type of lubricant, the amount of API in the tablet, and the mixing time for drug and the carrier powder did not have any significant effect on DE\textsubscript{10}% (p > 0.05).

**Optimization of tablets formulation**

DE\textsubscript{10}% was used for optimization of the tablets formulation. The software suggested formulation B9 as the optimum formulation which contained: Mg-stearate 1.2 mg as the lubricant, mixing time of 12 hours for mixing the carrier powder and the drug, Primojel 6 mg as the disintegrating agent, Avicel 20.8 mg as the filler, mannitol 90 mg as the carrier powder and 2 mg of the drug. The predicted amount of the DE\textsubscript{10}% for this formulation was 83.37% and its actual value as shown in Table III was 81.30%±2.50%. In other words, the Qualitech 4 software was able to predict the optimal formulation of the mucoadhesive sublingual tablets of lorazepam by a Taguchi design successfully.

**Conclusions**

The mucoadhesive sublingual tablets of lorazepam were designed and evaluated in vitro. Taguchi design was used successfully to optimize the formulation of the tablets. The tensile strength of the tablets was more affected by the mixing time of the drug with the carrier powder and the highest tensile strength was achieved by 24 hours of mixing. The porosity of tablets was more affected by the type of lubricant and the highest porosity was seen in tablets prepared by Mg-stearate. The type of disintegrating agent was the most effective factor on the disintegration time and DE\textsubscript{10}% of the tablets and Primojel containing tablets showed lower disintegration time and DE\textsubscript{10}% than Kollidone. The drug content of the tablets was the most effective parameter on the bioadhesion of the tablets and lower amounts of API showed more bioadhesion. The in vitro tests showed suitable properties of the designed tablets. Nevertheless future in vivo studies are needed to confirm the good bioadhesion effects that were seen in vitro and also the pharmacokinetic studies for studying the enhancement in the bioavailability of the drug in comparison to the usual marketed tablets.

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**References**