Brain Targeted Intranasal Zaleplon Solid Dispersion in Hydrophilic Carrier System; 2 3 Full Factorial Design and In-vivo Determination of GABA Neurotransmitter

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Brain-targeted intranasal zaleplon solid dispersion in hydrophilic carrier system; $2^3$ full-factorial design and in vivo determination of GABA neurotransmitter

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KEYWORDS
Zaleplon; intranasal; solid dispersion; brain targeting; GABA measurement

ABSTRACT
Intranasal zaleplon solid dispersion was formulated to enhance the solubility, bioavailability and deliver an effective therapy. Zaleplon belongs to Class II drugs, and undergoes extensive first-pass metabolism after oral absorption exhibiting 30% bioavailability. A $2^3$ full-factorial design was chosen for the investigation of solid dispersion formulations. The effects of different variables include drug to carrier ratio (1:1 and 1:2), carrier type (polyethylene glycol 4000 and poloxamer 407), and preparation method (solvent evaporation and freeze drying) on different dissolution parameters were studied. The dependent variables determined from the in vitro characterization and their constraints were set as follows: minimum mean dissolution time, maximum dissolution efficiency and maximum percentage release. Numerical optimization was performed according to the constraints set based on the utilization of desirability functions. Differential scanning calorimetry, infrared spectroscopy, X-ray diffraction and scanning electron microscopy were performed. Ex vivo estimation of nasal cytotoxicity and assessment of the GABA neurotransmitter level in plasma and brain 1 h after nasal SD administration in rabbits compared to the oral market product were conducted. The selected ZP-SD, with a desirability 0.9, composed of poloxamer 407 at drug to carrier ratio 1:2 successfully enhanced the bioavailability showing 44% increase in GABA concentration than the marketed tablets.

Introduction
Zaleplon (ZP) is a pyrazolopyrimidin derivative [1] hypnotic drug indicated for the short-term management of insomnia, without the risk of dependence or rebound insomnia upon discontinuation [2]. It also possesses potent anticonvulsant activity against pentylenetetrazole- and electroshock-induced convulsions [3]. The total dose of ZP is 10 mg before bedtime for healthy patients, while elderly, debilitated patients or those with mild-to-moderate hepatic impairment should be given 5 mg [4]. ZP is a full agonist for the benzodiazepine z1-receptor located on γ-aminobutyric acid type A (GABAA) receptor ionophore complex in the brain, with lower affinity for the z2 and z3-subtypes. It selectively enhances the release of GABA neurotransmitter, which is similar in action but more selective than benzodiazepines. Its selectivity for the z1-receptor rather than z2-receptor, is expected to provide sedative action with reduced effects on cognition and psychomotor function.

Even though, ZP is rapidly and completely absorbed after oral administration, it undergoes extensive first-pass hepatic metabolism after absorption, with only 30% of ZP being systemically available [5]. ZP attains peak concentration (Cmax) within 1.1 h (tmax) approximately after administration, with terminal elimination half-life of 1 h [6]. The ultra-short half-life gives ZP a unique advantage over other hypnotics as it lacks next day residual effects on driving and other performance related skills [7,8]. In addition, ZP belongs over other hypnotics as it lacks next day residual effects on driving life of 1 h [6]. The ultra-short half-life gives ZP a unique advantage approximately after administration, with terminal elimination half-life of 1 h [6].

Therefore, our aim was to formulate intranasal ZP solid dispersion (ZP-SD) to improve the solubility, dissolution and the bioavailability of the poorly water-soluble ZP where, intranasal formulation bypasses the first-pass effect and ensure brain targeting. Many techniques had been used to increase ZP solubility by formulating ZP as solid dispersions. Waghmare et al. [11] reported ZP solubility enhancement via solid dispersion preparation using various hydrophilic polymers such as Leutrol-F68, PVP-K30 and PEG-6000 with the aid of solvent evaporation method. Popescu et al. [12] reported the solubility enhancement of ZP using complexation technique with cyclodextrin and modified cyclodextrins namely methyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin by freeze-drying and spray drying methods. Abdelbary et al. [13] used different hydrotropic agents to improve ZP solubility via SD preparation. However, all the previous work aimed at formulating oral ZP SD tablets. To our knowledge, this is the first work to use solid dispersion intranasally using poloxamer 407 and polyethylene glycol 4000 as carriers for ZP-SD preparation.

Intranasal drug delivery system is used for both topical and systemic therapies and is now presented as an alternative to oral and parenteral routes [14]. There are two main mechanisms for absorption through the nasal mucosa and reaching the brain tissues; either it can be absorbed from the olfactory neural cells directly to
the brain tissues or it can be absorbed from the highly vascular respiratory region into systemic circulation. Intranasal drug delivery system bypass the first-pass metabolism and is directed to the targeted organ as brain tissue or cerebrospinal fluid [15]. The anatomical structure of the nose permits systemic drug delivery due to its high permeability, high vasculature which makes a speedy onset of action, and nearly neutral environment which overcome the limitation of oral route and duplicate the benefit of intravenous route. Moreover, it is a noninvasive route that can be administered by the patient with a higher degree of adherence and compliance than the intravenous route [14].

Materials and methods

ZP was a kind gift from Al Andalous for Pharmaceutical Industries (6th of October city, Egypt). Sleep aid[^5] 5 mg tablets was purchased from October Pharma S.A.E, 6th of October city, Egypt. Poloxamer 407 was kindly provided by BASF SE (Carl-Bosch-str. 0.3867056, Ludwigshafen, Germany). Polyethylene glycol-4000 (PEG-4000) were purchased from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). All other chemicals and solvents were of analytical grade and used as received.

Preparation of ZP-SD

A 2^4 full-factorial design was chosen for predicting and studying the experimental trials to choose the optimized formulation using Design-Expert™ software (version 7, Stat-Ease Inc., Minneapolis, MN) as shown in (Table 1). ZP-SD preparation was performed by two different techniques, namely freeze-drying and solvent evaporation [16]. Poloxamer 407 and PEG-4000 were the carriers investigated. To evaluate the effect of carrier amount on the formation of solid dispersion two different drug to carrier ratio (1:1 and 1:2) were tested. The compositions of the prepared ZP-SD are listed in (Table 2).

<table>
<thead>
<tr>
<th>Factors (independent variables)</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Drug to carrier ratio</td>
<td>1:1</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
</tr>
<tr>
<td>B: Carrier type</td>
<td>Poloxamer 407</td>
</tr>
<tr>
<td></td>
<td>PEG-4000</td>
</tr>
<tr>
<td>C: Preparation method</td>
<td>Freeze drying</td>
</tr>
<tr>
<td></td>
<td>Solvent evaporation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Responses (dependent variables)</th>
<th>Desirability constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1: MDT</td>
<td>Minimize</td>
</tr>
<tr>
<td>Y2: Q 60%</td>
<td>Maximize</td>
</tr>
<tr>
<td>Y3: Dissolution efficiency</td>
<td>Maximize</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug:carrier ratio</th>
<th>Carrier type</th>
<th>Preparation method</th>
<th>Drug content (%)</th>
<th>MDT ± SD</th>
<th>Q60 (%)</th>
<th>D.E. (%)</th>
<th>Release order</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diffusion</td>
</tr>
<tr>
<td>F1</td>
<td>1:1</td>
<td>Poloxamer 407</td>
<td>Solvent evaporation</td>
<td>100.3 ± 0.01</td>
<td>23.87 ± 0.2</td>
<td>40.21 ± 0.02</td>
<td>25.20 ± 0.001</td>
<td>Diffusion</td>
</tr>
<tr>
<td>F2</td>
<td>1:1</td>
<td>Poloxamer 407</td>
<td>Freeze drying</td>
<td>105.1 ± 0.2</td>
<td>11.95 ± 0.5</td>
<td>79.66 ± 0.2</td>
<td>63.79 ± 0.01</td>
<td>Diffusion</td>
</tr>
<tr>
<td>F3</td>
<td>1:2</td>
<td>Poloxamer 407</td>
<td>Solvent evaporation</td>
<td>98.43 ± 0.7</td>
<td>7.17 ± 0.8</td>
<td>100.61 ± 7.1</td>
<td>88.64 ± 0.1</td>
<td>First order</td>
</tr>
<tr>
<td>F4</td>
<td>1:2</td>
<td>Poloxamer 407</td>
<td>Freeze drying</td>
<td>98.9 ± 0.7</td>
<td>7.41 ± 0.3</td>
<td>100.61 ± 7.1</td>
<td>88.64 ± 0.1</td>
<td>First order</td>
</tr>
<tr>
<td>PM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.46 ± 1.0</td>
<td>59.18 ± 1.5</td>
<td>46.87 ± 0.002</td>
<td>–</td>
</tr>
<tr>
<td>F5</td>
<td>1:1</td>
<td>PEG-4000</td>
<td>Solvent evaporation</td>
<td>97.26 ± 0.04</td>
<td>15.98 ± 0.6</td>
<td>75.14 ± 0.5</td>
<td>55.12 ± 0.003</td>
<td>Diffusion</td>
</tr>
<tr>
<td>F6</td>
<td>1:1</td>
<td>PEG-4000</td>
<td>Freeze drying</td>
<td>100.34 ± 0.01</td>
<td>16.44 ± 3.7</td>
<td>66.35 ± 4.4</td>
<td>47.99 ± 0.001</td>
<td>Diffusion</td>
</tr>
<tr>
<td>F7</td>
<td>1:2</td>
<td>PEG-4000</td>
<td>Solvent evaporation</td>
<td>98.43 ± 0.7</td>
<td>7.17 ± 0.8</td>
<td>100.61 ± 7.1</td>
<td>88.64 ± 0.1</td>
<td>First order</td>
</tr>
<tr>
<td>F8</td>
<td>1:2</td>
<td>PEG-4000</td>
<td>Freeze drying</td>
<td>98.9 ± 0.7</td>
<td>7.41 ± 0.3</td>
<td>100.61 ± 7.1</td>
<td>88.64 ± 0.1</td>
<td>First order</td>
</tr>
<tr>
<td>PM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.74 ± 1.9</td>
<td>58.54 ± 1.5</td>
<td>46.73 ± 0.001</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2. Composition and in vitro characterization of solid dispersion prepared formulae (in comparison to pure drug and physical mixtures.

![Data table]

In vitro release study

The dissolution profiles of pure drug, the prepared SD formulae, and their corresponding drug–carrier physical mixtures were studied using USP dissolution tester (DIS 6000, Copley Scientific Ltd, Nottingham, UK). An accurately weighed amount equivalent to 5 mg ZP was placed in a rotating basket covered by standard mesh packet [18]. Dissolution was carried out in 250 ml of phosphate buffer pH 6.27 at 50 rpm, and 37 ± 0.5 °C. About 3 ml samples were withdrawn at regular time intervals of 5, 10, 15, 20, 25, 30, 45 and 60 min. The volume of dissolution medium was maintained constant by replacing aliquots withdrawn with 3 ml of buffer to maintain sink conditions. Withdrawn samples were filtered through 0.45 μm membrane filter, suitably diluted and analyzed spectrophotometrically using UV spectrophotometer (Shimadzu, UV-2401 PC, North South Wales, Australia) at λmax 232 nm.

Freeze-drying method

The drug was dissolved in methanol and the carrier was dissolved in distilled water. The drug solution was poured all at once on the carrier dispersion and vortexed for 1 min then dried by freeze-drying technique (Lyophilizer: SM 16 PDM, Savant, Holbrook, NY). The formed solid mass was scratched and then sieved (40 mesh) [17].

Solvent evaporation method

The drug–carrier dispersions were prepared by applying the above procedure then dried by Rotavap technique (Rotavap: Heidolph, Laboroto 4000 efficient, USA) under vacuum at 70 °C. The formed solid mass was scratched and then sieved (40 mesh) [17].

In vitro characterization of ZP-SD formulations

Determination of drug content

An amount of ZP-SD equivalent to 5 mg of ZP was dissolved in 100 ml methanol and then filtered through 0.45 μm membrane filter. The drug content was determined in duplicate spectrophotometrically using UV spectrophotometer (Shimadzu, UV-2401 PC, North South Wales, Australia) at λmax 232 nm.

In vitro release study

The dissolution profiles of pure drug, the prepared SD formulae, and their corresponding drug–carrier physical mixtures were studied using USP dissolution tester (DIS 6000, Copley Scientific Ltd, Nottingham, UK). An accurately weighed amount equivalent to 5 mg ZP was placed in a rotating basket covered by standardized mesh packet [18]. Dissolution was carried out in 250 ml of phosphate buffer pH 6.27 at 50 rpm, and 37 ± 0.5 °C. About 3 ml samples were withdrawn at regular time intervals of 5, 10, 15, 20, 25, 30, 45 and 60 min. The volume of dissolution medium was maintained constant by replacing aliquots withdrawn with 3 ml of buffer to maintain sink conditions. Withdrawn samples were filtered through 0.45 μm membrane filter, suitably diluted and analyzed spectrophotometrically using UV spectrophotometer at λmax 232 nm. The mean drug release percentages (±SD) were plotted versus time. The mean dissolution time (MDT) was calculated from Equation (1):

$$ MDT = \frac{\sum_{i=1}^{n} T_{mid} \Delta M}{\sum_{i=1}^{n} \Delta M} $$

Where, i is the dissolution sample number, n is the number of dissolution sampling times, $T_{mid}$ is the midpoint between times $T_i$ and $T_{i-1}$, and $\Delta M$ is the amount of ZP dissolved between times $T_i$ and $T_{i-1}$ [19].

Values are mean ± standard deviation of three samples.
The dissolution efficiency values were calculated as proposed by Khan and Rhode and is defined in Equation (2) [20].

\[
\text{Dissolution efficiency} \; (\%) = \frac{\int_{t_1}^{t_2} y \, dt}{y_{100} (t_2 - t_1)} \times 100
\]

It is the area under the dissolution curve obtained by trapezoidal rule between time points of \( t_1 \) and \( t_2 \), expressed as a percentage of the curve at maximum dissolution, \( y_{100} \) over the same period. One-way analysis of variance (ANOVA) (post hoc, least significant difference (LSD) test) was carried out to compare their means using SPSS 19.0 \(^\circ\) software (Released 2010. IBM SPSS Statistics for Windows, Version 19.0. IBM Corp., Armonk, NY) at a significance level \( \alpha = 0.05 \).

**Kinetic analysis of the dissolution studies**

The data obtained from the dissolution experiments were analyzed to find out the kinetics of drug dissolution to determine the dissolution model, the in vitro dissolution data were analyzed according to zero order, first order and diffusion-controlled Hibiguchi mechanism. The preference of a certain dissolution order was based on the determination of correlation coefficient \( (R^2) \). Where the highest coefficient of determination is preferred for the selection of the order of release.

**Statistical analysis**

Design-Expert software was used for the generation and evaluation of the statistical experimental design. Means were compared by ANOVA-factorial. Significance level was set at \( \alpha = 0.05 \). Suitable regression models were driven to enable navigation of the experimental space [21]. Response surface methodology and multiple response optimization were used to search for an optimized formula [22].

Numerical optimization [23,24] was performed using the statistical program according to the constraints listed in Table 1. The simultaneous optimization technique described by Derringer and Suich in 1980 was chosen for optimization of the responses. This method is based on the utilization of desirability functions.

**Solid state characterization of the optimized ZP-SD**

The chosen formula was subjected to thermal analysis using differential scanning calorimetry (DSC), Fourier-transformed infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD) and was morphologically investigated using scanning electron microscope (SEM).

**Differential scanning calorimetry**

Differential scanning calorimetry was performed for the pure drug, poloxamer 407, physical mixture (PM) and the selected formula (F3) using differential scanning calorimeter (Model DSC-50; Shimadzu Corporation, Kyoto, Japan). Samples of 10 mg were placed in flat-bottomed aluminum pan under atmosphere of nitrogen at flow rate 25 ml/min. The hold temperature was set at 350 °C and the temperature acceleration rate was 10 °C/min.

**Fourier-transformed infrared spectroscopy**

The FT-IR spectra of the selected formula (F3) was recorded using IR spectrophotometer (Shimadzu IR-435). The IR spectrum was compared to that of pure drug, poloxamer 407 and physical mixture to investigate the probability of chemical interactions between ingredients of the selected formula (F3). The scanning was performed in potassium bromide discs within a wave number of 4000–400 cm\(^{-1}\).

**Powder X-ray diffraction**

The X-ray diffraction pattern for the pure drug, Poloxamer 407, PM and formula (F3) were recorded at room temperature using X-ray diffractometer: (model XGEN-4000, X1 advanced diffraction systems; Scintag Corp., Sunnyvale, CA) at 45 kV and 40 mA. The scanning rate was 2 °C/min over a diffraction angle (2θ) range of 0–50 °C.

**Scanning electron microscopy**

The surface morphology of the selected formula was examined by means of a SEM (JSM-6390 LV, JEOL, Tokyo, Japan). The powders were fixed on a brass stub using double-sided adhesive tape and then made electrically conductive by coating in a vacuum chamber with a thin layer of gold for 30 s. The pictures were taken at an excitation voltage of 20 kV.

**In vivo studies**

**Preparation of ZP nasal suspension.** Of SD, 30 mg which is equivalent to 10 mg ZP was dispersed in 1 ml phosphate buffer pH 6.27 and it was administered as nasal suspension. Rabbits administered low (0.625 mg/2 kg) and high doses (1.25 mg/2 kg) of SD nasal suspension equivalent to 62.5 μl and 125 μl, respectively.

**Assessment of GABA level in rabbit plasma and brain.** ZP appears to increase the efficiency of GABAergic synaptic inhibition. It does not substitute for GABA but appears to enhance GABA’s effects allosterically (as positive allosteric modulators) without directly activating GABA \( \alpha \)-receptors or opening the associated chloride channels. They appear to increase the duration of the GABA-gated chloride channel openings [25]. Therefore, assessment of GABA levels in rabbits’ plasma and brain would ensure that ZP reached its site of action and was able to bind to \( \alpha-1 \) GABA receptor and exert its mechanism of action. The efficiency of the selected SD to deliver the drug across the blood–brain barrier was evaluated on rabbits. Thirty healthy adult male albino rabbits weighing between 1900 and 2000 g contributed in the study. The protocol of the study was reviewed and approved (PI 1197) by the institutional review board; Research Ethics Committee-Faculty of Pharmacy, Cairo University (REC-FOPCU). The rabbits were housed three per cage at room temperature with free access to food and water with a 12-h light–dark cycle. A parallel design was conducted where the animals were divided into five groups and each group contains six rabbits Group 1: Intranasal Saline solution, Group 2, 3: Intranasal SD receiving low (SDL) and high doses (SDH) and Group 4, 5: Oral Sleep Aid \(^\circ\) marketed product receiving low (SL) and high doses (SH). The low and high doses were 0.125 and 0.625 mg/2 kg rabbit [26]. For intranasal formulations, the conscious rabbits were held from the back in a slanted position. The formulations were administered at one opening of the nostrils (the other was kept as negative control in histopathological study). The procedure was performed gently, allowing the animals to inhale all the preparation. As for the oral formulation, the conscious rabbits were made to swallow the predetermined dose of Sleep Aid \(^\circ\) using a specialized oral applicator, then 5 ml water was administered post dose. After 1 h of dose administration, Rabbits from the five groups were humanely sacrificed. Blood samples were collected into the heparinized tubes as an anticoagulant and then centrifuged at 6000 rpm for 15 min using Cooling centrifuge.
was adjusted at 56°C till analysis. GABA concentration in plasma was determined by GABA Elisa Kits [13]. The brain was dissected, washed twice using normal saline, made free from adhering tissue/liquid and weighed. Aliquots of ice cold saline solution were added in a ratio 1:1. The organ was then homogenized and finally GABA concentrations were determined using GABA Elisa Kits. Statistical analysis between the groups received market product (Sleep Aid®) and SD formula (F3) at different doses was computed by unpaired Student’s t-test.

Estimation of nasal toxicity. The nasal-cavity mucosa of the nostril of the sacrificed male albino rabbits in which low and high doses ZP-SD were administered, were compared with negative control nasal-cavity mucosa (the other nostril) of the same rabbit. Within 1 h of the sacrifice of the animal, the nasal cavity was fully exposed by a longitudinal incision through the lateral wall of the nose while avoiding the damage of the septum. Following, the mucosa was carefully removed and the pieces were washed with distilled water and preserved in 10% formalin solution [27]. The histopathological studies were conducted according to the protocol described by Bancroft et al. [28]. Briefly, the samples were dehydrated by treatment with serial dilutions of methyl alcohol, ethyl alcohol, and absolute ethyl alcohol, respectively. Specimens were cleared in xylene and embedded in paraffin in the oven. The temperature of the oven was adjusted at 56°C and the samples were kept for 24 h. Paraffin-beeswax tissue blocks were sectioned by a sledge microtome. The obtained tissue sections (3–4 μm thickness) were collected, de-paraffinized, stained by hematoxylin and eosin, and examined under a light microscope.

Results and discussion

In vivo characterization of ZP-SD formulations

Determination of drug content

The mean values of ZP content in different carrier systems ranged from 96.76 to 105.1% as shown in (Table 2) confirming the absence of drug loss during the dispensing procedures.

In vivo release study

Drug to carrier ratio, carrier type, and preparation method had a significant three factors interaction (3FI) on MDT, Q60, and D.E.%, respectively. (Figure 1) shows the in vitro release profiles of ZP from solid dispersion formulae (F1–F8). (Table 2) shows the percentage of drug dissolved at 60 min (Q60), the dissolution efficiency at 60 min (D.E.%) and the MDT for the solid dispersion formulae (F1–F8), their corresponding physical mixtures and the pure drug. All binary systems showed higher Q60 and D.E.% than their corresponding physical mixtures and pure drug as well. This indicates that improving the dissolution of the drug from the different formulae was due to formation of solid dispersion not only due to wetting effect of the carrier on the drug. The post Hoc LSD test showed a significant increase in MDT, Q60, and D.E.% than pure ZP. On one hand Formulae 1–8 were arranged in ascending order according to their MDT into F3 < F4 < F8 < F5 < F6 < F7, where F3, F4, and F8 showed insignificant difference in the MDT from each other, followed by F2 which showed insignificant difference from F8 only. As for F1, a significant difference is observed from all formulae. F5, F6, and F7 showed significant increased MDT than other formulae and insignificant difference in between them. A significant decrease in MDT was observed as the ratio increased from 1:1 to 1:2, except for PEG-400 with solvent evaporation technique where non-significant change was observed. On the other hand, Formulae 1–8 were arranged in descending order according to their Q60: F3 > F4 > F1 > F5 > F7 > F2 > F6 > F8, where F3 showed significant increase in the percentage of drug released after 60 min than other formulae, followed by F4, then F1 and F5 were insignificantly different. F7, F2, and F6 were insignificantly different from each other but significantly higher than F8, which in turn showed significantly higher percentage release than pure drug. As for D.E.%, Formulae 1–8 were arranged in descending order: F3 > F4 > F1 > F2 > F5 > F7 > F8 > F6 > F8, where F3 and F4 showed significant increase in D.E.% than other formulae, then F1 and F2 were insignificantly different. F5 and F8 were insignificantly different from each other but significantly higher than F7 and F6, which in turn showed significantly higher dissolution efficiency than pure drug. The improvement in dissolution of ZP from solid dispersion may be accredited to decrease in the drug crystallinity plus its
molecular dispersion in hydrophilic carrier matrices. As soluble carrier dissolves, the insoluble drug gets exposed to dissolution medium in the form of very fine particles for rapid dissolution [29,30], in addition to carrier hydrophilicity, surfactant property, increased wettability and dispersibility [31]. Moreover, a significant positive effect was observed on Q60, and D.E.% where an increase in the percentage release occurred as the weight fraction of the carrier increase in case of Poloxamer 407 while PEG-4000 showed non-significant effect on the percentage of drug released from both ratios (as stated earlier by the post hoc LSD test). Solid dispersions prepared using Poloxamer 407 showed a significant difference than those using PEG-4000, where Poloxamer 407 resulted in decreased MDT and increased Q60 and D.E.% than PEG-4000. This might be attributed to more wetting and solubilizing effect of Poloxamer compared to PEG-4000 resulting in increases in surface available for dissolution by reducing interfacial tension between hydrophobic drug and dissolution medium [11,32]. In addition to the gelling tendency of aqueous solution of PEG-4000 which might contribute to the increased MDT and decreased Q60 and D.E.% compared to Poloxamer 407 [33]. As for the preparation method, it was clear that solid dispersions prepared by solvent evaporation method presented more rapid and enhanced dissolution profile than those prepared by freeze-drying method, as they displayed significantly increased Q60 and D.E. whereas a non-significant effect was detected on the MDT. An assumption is made that solvent evaporation technique results in uniform distribution of drug within the carriers leading to such findings [11].

Kinetic analysis of dissolution studies

Kinetic analysis according to zero order, first order and Higuchi diffusion model of release showed that the release of ZP from all formulae fitted better with first order and Higuchi diffusion model rather than zero order release. The best fitting model was first order release with formulae (F3, F5, and F7) and Higuchi diffusion model with the other formulae as shown in (Table 3).

**Statistical analysis**

Each response that was mentioned in (Table 1), was analyzed individually and fitted 3FI model using linear regression. Sequential model sum of squares, lack of fit test and model summary statistics were used to elucidate the best model to describe the relation between the response under question and the variables studied. The highest order unaliased model with highest prediction R² and model sum of squares, lack of fit test and model summary statistics were used to elucidate the best model to describe the relation between the response under question and the variables studied. The highest order unaliased model with highest prediction R² and lowest PRESS was the one of choice. Further improvement of the chosen model was done by model reduction, by removing any non-significant model terms that are not needed to support hierarchy.

**Table 3.** Kinetic analysis of the dissolution data of solid dispersion formulae (F1–F8) in comparison to pure drug.

<table>
<thead>
<tr>
<th>Formulae</th>
<th>R²</th>
<th>Release order</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZP</td>
<td>0.9782</td>
<td>0.9921</td>
<td>0.9977</td>
<td>Higuchi diffusion</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>0.8682</td>
<td>0.9305</td>
<td>0.9362</td>
<td>Higuchi diffusion</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>0.5969</td>
<td>0.6512</td>
<td>0.7383</td>
<td>Higuchi diffusion</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>0.745</td>
<td>0.973</td>
<td>0.8558</td>
<td>First order</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>0.8631</td>
<td>0.8994</td>
<td>0.9053</td>
<td>Higuchi diffusion</td>
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<td>F5</td>
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<td>0.9838</td>
<td>0.9811</td>
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<td>F6</td>
<td>0.7876</td>
<td>0.8292</td>
<td>0.8846</td>
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<td>F7</td>
<td>0.9572</td>
<td>0.9731</td>
<td>0.9687</td>
<td>First order</td>
<td></td>
</tr>
<tr>
<td>F8</td>
<td>0.6814</td>
<td>0.7277</td>
<td>0.8103</td>
<td>Higuchi diffusion</td>
<td></td>
</tr>
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</table>

**Elucidation of optimized formula**

After generating the final polynomial equations relating the dependent and independent variables, optimization was done for the three responses studied. Numerical optimization searched the design space using the final models created for each studied response. It aimed at finding factors’ levels that meet the constraints for different responses. Formula 3 was chosen with overall desirability equal to 0.9, which means that it satisfies the constraints previously stated in (Table 1).

**Solid state characterization of the optimized ZP-SD**

The physicochemical properties of the optimized formula were studied to ensure the formation of solid dispersion.

**Differential scanning calorimetry**

The DSC thermograms of ZP, Poloxamer 407, PM and the selected formula F3 are represented in Figure 2(A). The DSC thermogram of ZP alone shows endothermic peak at 189.77 °C. The sharp peak is indicative of strong crystal lattice energy which can be considered as one of the factors responsible for lower ZP aqueous solubility [34]. The DSC thermogram of Poloxamer 407 showed sharp endothermic peak at 60.51 °C corresponding to its melting point which agrees with the finding of Newa et al. [35]. The sharp melting point peak of the drug appeared but with decreased intensity in the PM. The sharp melting point peak of the drug greatly decreased in the DSC thermogram of formula F3 indicating that ZP was molecularly dispersed and suggesting the conversion of ZP from crystalline into amorphous form confirming the increase in ZP solubility and enhanced dissolution profile. Additionally, the peak of Poloxamer 407 in F3 was found to be shifted to lower value i.e. 57.12 °C indicating solid–solid phase transition [36].

**FT-infrared spectroscopy**

The infrared spectrum of ZP, as shown in (Figure 2(B1)), exhibited the characteristic bands (marked by red arrows) at 3074.53 cm⁻¹ corresponding to C–H aromatic, 2981.95 cm⁻¹ attributed to C–H aliphatic, and in addition to the appearance of a band at 2233.57 cm⁻¹ assigned for cyanide (C≡N) stretching, a band at 1651.07 cm⁻¹ corresponding to amide carbonyl group C = O. In addition to the appearance of a band at 1612.49 cm⁻¹ attributed to C = N bending and two bands at 1222.87 and 1550.77 cm⁻¹ corresponding to C–N and C = C aromatic, respectively. For poloxamer 407, IR spectrum (Figure 2(B2)) showed characteristic strong absorption band at 3081–2900 cm⁻¹ corresponding to C–H aliphatic and a broad band at 3400–3550 cm⁻¹ attributed to O–H alcoholic. PM IR spectrum (Figure 2(B3)) maintained the characteristic bands of both Poloxamer 407 and ZP confirming the absence of any chemical interaction with the absence of formation of new peaks [37]. Formula F3 IR spectrum (Figure 2(B4)) showed decreased intensity in the characteristic bands of ZP (marked by red arrows) as shown. Most peaks of ZP were found to be smoothened indicating strong physical interaction with Poloxamer 407 [38].

**Powder X-ray diffraction**

The X-ray diffraction patterns for the pure drug, poloxamer 407, PM and F3 are shown in (Figure 2(C)). The PXRD pattern of the pure drug exhibited sharp and intense peaks which is indicative of its...
strong crystalline nature with three prominent peaks of high intensity at 2θ = 10.40°, 14.66° and 14.41° in addition to other eight less prominent peaks at 2θ = 14.102°, 18.793°, 17.19°, 19.01°, 20.02°, 25.68°, 26.63° and 29.38°. The PXRD of poloxamer 407 showed three prominent peaks at 2θ = 19.40°, 23.34° and 23.71°. A comparison of PXRD of pure drug with that of PM showed reduction in peak intensity but still the drug retained some of its crystallinity. Considering F3 SD, PXRD showed sharp decrease in the intensity of some peaks of the drug and disappearance of the others which could be attributed to the destruction of the drug crystal lattice because of progressive amorphization [39] which indicate that the drug was molecularly entrapped inside the network, i.e. in a physical state readily available to the dissolution process in a release medium from formula F3. Hence, the increase in dissolution of the F3 could be due to the amorphous form of the drug. An amorphous or metastable form dissolves at the fastest rate because of its higher internal energy and greater molecular motion which
enhance thermodynamic properties relative to crystalline materials [40,41].

Scanning electron microscopy
SEM of formula F3 using 40,000× magnification power revealed the surface topography studies performed. The solid dispersion was observed as irregular shaped agglomerates of

Figure 3. Photomicrographs of the rabbit nasal mucosa treated with A1, A2: normal saline, B1, B2: SD low dose, C1, C2: SD high dose. Where 1: H 1E X16, and 2: H 1E X40 magnification power.

<table>
<thead>
<tr>
<th>Formulæ</th>
<th>GABA concentration in Plasma (ng/ml)</th>
<th>GABA concentration in brain tissue (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1.98 ± 0.12</td>
<td>65 ± 6</td>
</tr>
<tr>
<td>F3 ZP-SD (Low dose)</td>
<td>8.23 ± 0.40</td>
<td>181 ± 4</td>
</tr>
<tr>
<td>F3 ZP-SD (High dose)</td>
<td>12.90 ± 0.65</td>
<td>342 ± 15</td>
</tr>
<tr>
<td>Sleep Aid® (Low dose)</td>
<td>6.10 ± 0.54</td>
<td>125 ± 5</td>
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<tr>
<td>Sleep Aid® (High dose)</td>
<td>10.02 ± 0.44</td>
<td>237 ± 9</td>
</tr>
</tbody>
</table>
the drug in the poloxamer matrix. It appeared in the form of smooth, uniform, and homogeneously mixed mass (Figure 2(D)) [32].

**In vivo studies**

**Estimation of nasal toxicity**

The negative control group, administrated saline, showed no histopathological alteration and normal histological structure of the lining pseudostratified columnar epithelium with the underlying lamina propria and cartilaginous structure as recorded in Figure 3(A1,A2). Similarly, SD formula F3 at low and high doses showed no histopathological alteration in the mucosa as recorded in Figure 3(B1,B2) for SD low dose and Figure 3(C1,C2) for SD high dose.

**Assessment of GABA level in rabbit plasma & brain**

ZP acts on (GABAA) receptors in the central nervous system (CNS) by binding with the α1, β2 and γ2-subunits of GABAA receptors resulting in an increase in GABA concentration [42]. Hence, the therapeutic effectiveness of ZP depends upon the availability of GABA receptors in the brain. The plasma and brain GABA level of groups receiving ZP-SD formula (F3) at different doses (0.625 and 1.25 mg/2 kg) were significantly higher than those receiving the marketed product Sleep Aid tablet. Thus, intranasal ZP-SD may be a promising replacement for the oral Sleep Aid tablet.

**Conclusion**

Intranasal ZP-SD formula (F3) composed of Poloxamer 407 and ZP in drug to carrier ratio 1:2 successfully prepared using solvent evaporation showed the highest percentage of drug released (100.61 ± 7.16) and dissolution efficiency (88.64 ± 0.08) with minimum MDT (7.17 ± 0.85). This promising in vitro results comes in agreement with the in vivo studies which showed significant 44% increase in plasma and brain GABA levels compared to the marketed product Sleep Aid®. Thus, intranasal ZP-SD may be a promising replacement for the oral Sleep Aid® tablet.

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**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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**References**


