Research paper

Floating lipid beads for the improvement of bioavailability of poorly soluble basic drugs: In-vitro optimization and in-vivo performance in humans

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ABSTRACT

The challenge in developing oral drug delivery systems of poorly soluble basic drugs is primarily due to their pH dependent solubility. Cinnarizine (CNZ), a model for a poorly soluble basic drug, has pH dependent solubility; where it dissolves readily at low pH in the stomach and exhibits a very low solubility at pH values greater than 4. It is also characterized by a short half life of 3–6 h, which requires frequent daily administration resulting in poor patient compliance. In an attempt to solve these problems, extended release floating lipid beads were formulated. A 2^4 full factorial design was utilized for optimization of the effects of various independent variables; lipid:drug ratio, % Pluronic F-127, % Sterotex, and Gelucire 43/01:Gelucire 50/13 ratio, on the loading efficiency and release of CNZ from the lipid beads. In-vivo pharmacokinetic study of the optimized CNZ-lipid beads compared to Stugeron® (reference standard) was performed in healthy human volunteers. A promising approach for enhancing the bioavailability of the poorly soluble basic drug, CNZ, utilizing novel and simple floating lipid beads was successfully developed. Zero order release profile of CNZ was achieved for 12 h. Mean AUC0–24 of the optimized CNZ-loaded lipid beads were 4.23 and 6.04 times that of Stugeron® tablets respectively.

1. Introduction

The development of oral drug delivery systems for poorly soluble basic drugs is problematic because of their pH dependent solubility. Poorly water soluble basic drugs are very sensitive to pH changes, and following oral administration and dissolution in the acidic stomach environment, they tend to precipitate upon gastric emptying to higher pH medium in the intestine, leading to compromised or erratic oral bioavailability [1,2].

Cinnarizine (CNZ) is a lipophilic drug with partition coefficient of logP = 5.8. It is a weak base with pK_a1 = 2 and pK_a2 = 7.5 having pH-dependent solubility. It exhibits higher solubility at low pH values (0.29 mg/ml in 0.1 N HCl) and lower solubility at higher pH (0.002 mg/ml in phosphate buffer pH 7.2) [3]. CNZ was chosen as a model compound representing poorly soluble basic drugs.

CNZ is a piperazine derivative with antihistaminic, sedative and calcium channel blocker. It is used in the treatment of nausea and vertigo caused by Meniere disease and other vestibular disorders and for prevention and treatment of motion sickness. CNZ has a short half-life of 3–6 h [4]. The usual dose for vertigo and vestibular disorders is 25 or 30 mg three times daily [5,6] and the only commercially available dosage forms are immediate release tablets and capsules, hence frequent daily administration is required resulting in poor patient compliance.

Aiming to solve these problems, extended release floating drug delivery system for continuous delivery of CNZ in the stomach was proposed [7]. Not only for reducing frequency of drug administration, but also it was suggested that prolonged gastric retention with slow continuous drug release, could result in the enhancement of CNZ bioavailability, through reducing the expected drug

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; C_{max}, peak concentration; CNZ, Cinnarizine; DSC, differential scanning calorimetry; Gel, Gelucire; h, hour; IS, internal standard; LC-MS/MS, liquid chromatography mass spectroscopy; SEM, scanning electron microscope; T_{max}, time of peak concentration; XRD, X-ray diffraction.

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precipitation from its acidic solution upon gastric emptying and its contact with higher pH environment in the intestine.

Green technologies, which offer freedom from organic solvents, are preferred due to stringent global environmental concerns. Lipid-based sustained release matrix systems have emerged as promising and efficacious agents with wide spectrum of desired characteristics for effective drug delivery. They are characterized by low melt viscosity, thereby obviating the need of organic solvents for dissolving the drug, the absence of toxic impurities such as residual monomers catalyst and initiators, the potential biocompatibility and biodegradability, and prevention of gastric irritation by forming a coat around the drug were considered as the main advantages of lipid carriers compared to polymers [8,9].

The aim of this work was to statistically optimize a novel, simple approach utilizing floating, gastro-retentive, controlled release lipid beads, potentially suitable for once daily administration aiming to improve the low and erratic bioavailability for poorly soluble basic drugs (using CNZ as a model drug). A $2^4$ full factorial design was utilized for the optimization of the effects of the lipid:drug ratio, % Pluronic F-127, % Sterotex, and ratio between Gelucire 43/01:Gelucire 50/13 on drug loading and release extent from the prepared beads. The pharmacokinetic parameters obtained from the optimized CNZ lipid beads and Stugeron® as the marketed reference product were compared in healthy human volunteers.

2. Materials and methods

2.1. Materials

Cinnarizine HCl (CNZ) was supplied as a gift sample by Minapharm Pharmaceuticals Ltd. (Cairo, Egypt), Gelucire 43/01 (hard fat, melting point 43°C, HLB = 01) and Gelucire 50/13 (Stearoyl macrogol-32 glycerides, melting point 50°C, HLB = 13) were kindly obtained as gift samples from Gattefosse St Priest, Cedex, France, Sterotex NF (hydrogenated cotton seed oil, white solid powder, melting point 63°C) was kindly supplied by Abitec Corp. (Janesville, WI), Pluronic F-127 (Ethylene Oxide/Propylene Oxide Block Copolymer, melting point 56°C, HLB = 22) was provided by BASF (Ludwigshafen, Germany), and Hydrochloric acid (HCl) is of analytical reagent grade.

2.2. Preparation of Cinnarizine floating lipid beads

Floating lipid CNZ beads were prepared according to the method of Siepmann [10]. Briefly, the lipids were molten at 65°C, mixed well and then CNZ was dispersed. The molten dispersion containing the drug was then added to 100 ml pre-chilled water (4°C) at a rate of 5 ml/min via 23 gauge syringe and stirred at 100 rpm on a magnetic stirrer (model SP 72220-26, Barnstead/ Thermolyne, USA). Finally, the formed beads were filtered through Whatman 41, collected, and stored in glass vials.

2.3. Formulation of Cinnarizine floating lipid beads using 2$^4$ full factorial designs

A full factorial design ($2^4$) was employed in the formulation of CNZ floating lipid beads for the screening of the influence, and optimization of the four studied factors namely: Lipid (Gelucire 43/01, Gelucire 50/13 and Sterotex):drug ratio (A), percent of Pluronic F-127 (calculated using the total weight of beads including the drug) (B), percent of Sterotex (calculated from the lipid content) (C), and Gelucire 43/01:Gelucire 50/13 ratio (ratio in the remaining lipid content after calculating the amount of Sterotex) (D), each at two levels, as shown in Table 1. The observed responses were the percent of drug released after 1, 5, and 8 h ($Y_1$, $Y_2$, and $Y_3$, respectively) in addition to the percent of drug loaded in the beads ($Y_4$) using Minitab® software (version 16.1.1). Sixteen formulae were suggested and randomly arranged by the software and duplicate experimentation was carried out. The results of the observed responses were presented as mean ± standard deviation.

2.4. Evaluation of CNZ lipid beads

2.4.1. Floating behavior

The prepared beads (about 20 beads) were placed in 100 ml of 0.1 N HCl containing 0.02% Tween 20 at room temperature. Tween was added to mimic the wetting effect of the surfactants, naturally present in the gastrointestinal tract [11,12]. The mixture was stirred at 100 rpm on a magnetic stirrer. The time required to start floating, as well as time duration of floating was determined by visual observation.

2.4.2. In-vitro release study

The release of CNZ from the prepared floating beads was determined using USP paddle type (II) dissolution apparatus (Hanson Research, USA). A weighed amount of beads equivalent to 25 mg drug was placed in the dissolution vessel. Nine hundred milliliters of 0.1 N HCl containing 0.02% w/v Tween 20 was used as the dissolution medium [9]. The dissolution fluid was maintained at 37 ± 0.5°C at a rotation speed of 100 rpm. Five milliliter samples were withdrawn at the specified time intervals, filtered through a 0.2 μm Millipore filter, and the initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal. Samples were analyzed using a UV–visible spectrophotometer (Shimadzu, Japan) at 253 nm. The experiment was performed in duplicate.

2.4.3. Loading efficiency determination

CNZ content in lipid beads was determined by grinding accurately weighed 50 mg beads in 10 ml of 0.1 N HCl, followed by sonication at 70°C for 15 min and then, allowed to cool at room temperature. The lipid was solidified and the drug solution was filtered through Millipore filter 0.2 μm [13]. The samples were analyzed for drug content by a validated UV spectrophotometer method at 253 nm using UV-visible spectrophotometer (Shimadzu, Japan) after suitable dilutions. The experiment was performed in duplicate. The loading efficiency was calculated using the following equation:

\[
\text{Loading efficiency} \% = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100.
\]

<p>| Table 1 |</p>
<table>
<thead>
<tr>
<th>Factors (independent variables)</th>
<th>Level used</th>
<th>Constraints</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Lipid:drug ratio</td>
<td>2:1, 6:1</td>
<td>7% ≤ $Y_1$ ≤ 27%, target = 17%</td>
</tr>
<tr>
<td>B: % Pluronic</td>
<td>6%, 12%</td>
<td>32% ≤ $Y_2$ ≤ 52%, target = 42%</td>
</tr>
<tr>
<td>C: % Sterotex</td>
<td>0%, 2%</td>
<td>57% ≤ $Y_3$ ≤ 77%, target = 67%</td>
</tr>
<tr>
<td>D: Gelucire 43/01:Gelucire 50/13 ratio</td>
<td>5:1, 8:1</td>
<td>80% ≤ $Y_4$ ≤ 100%, maximize</td>
</tr>
</tbody>
</table>

2.4.4. Analysis of the factorial design

The first-order polynomial regression equations were generated between the factors and responses and described by the following equation:

\[ Y = b_0 + b_1 A + b_2 B + b_1C + b_2D + b_{12}AB + b_{13}AC + b_{14}AD + b_{23}BC + b_{24}BD + b_{134}ABC + b_{134}ACD + b_{234}BCD + b_{1234}ABCD \]

where \( Y \) is the dependent variable (response), \( b_0 \) is the intercept representing the arithmetic averages of all the quantitative outcomes of all experimental runs; \( b_1 \)–\( b_4 \) are the coefficients computed from the observed experimental values of \( Y \); and \( A, B, C, \) and \( D \) are the coded levels of factors. Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor represent the interaction between those factors. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries. A positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect of the factors [14].

2.4.5. Morphological examination and scanning electron microscope (SEM)

The morphology of the prepared beads was visualized using light microscopy. Optimized floating CNZ lipid beads were also investigated using scanning electron microscope Jeol (Jxa-840A, Japan) to investigate their surface morphology at different magnification powers before and after drug release. Samples were prepared by sticking the beads on a double adhesive tape, which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300 Å using a sputter coater [15].

2.4.6. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis was carried out using a DSC (Shimadzu, Japan) to evaluate any possible drug–lipid interaction. Thermograms of pure drug, individual excipients, physical mixtures (1:1 w/w) and optimized CNZ lipid beads were obtained. Samples of 2–6 mg were placed in aluminum pans and sealed. The pans were heated from 25 °C to 200 °C at a rate of 10 °C/min under nitrogen atmosphere.

2.4.7. X-ray diffraction (XRD) analysis

The X-ray diffraction study was carried out to characterize the physical form of CNZ in pure state, physical mixtures with the studied excipients (1:1 w/w) and in the optimized formula. The diffractograms were recorded at room temperature using Philips X-ray diffractometer with monochromator Cu-radiation (1.542 Å), at 40 kV, 35 mA. The diffractometer was equipped with a 2θ compensating slit, and was calibrated for accuracy of peak positions with silicon pellet. Samples were subjected to X-ray powder diffraction analysis in continuous mode with a step size of 0.02° and step time of 1 s over an angular range of 2–60° 2θ.

2.4.8. In-vivo study

2.4.8.1. Experimental design. A study was carried out to compare the pharmacokinetic parameters of CNZ from the optimized lipid beads containing 25 mg CNZ (test product) and the commercially available, immediate release Stugeron® tablets containing 25 mg CNZ (reference product), after single administration. Two treatments, two periods, randomized, and crossover design was carried out. Six male healthy human volunteers participated in the study and none of them were on any treatment one week before the study. The study was fully explained to the volunteers before starting the study. The protocol was approved by Cairo University Ethical Committee. An informed consent was signed by each volunteer after he had understood the aim of his participation in the study.

The two-period study was performed as follows: Period I, three human volunteers (group 1) administered Stugeron® tablets and the other three human volunteers (group 2) administered optimized CNZ lipid beads, equivalent to 25 mg CNZ. The volunteers were asked to fast for at least 10 h with free water access, till 1 h before treatments administration. Treatments were given with about 200 ml water. They were only allowed to take water after 2 h and a low calorie breakfast after 4 h from treatments administration. A one week washout period separated the two periods. In period II, the opposite treatment was administered.

The study was supervised by a physician who was responsible for the safety and collection of samples of the volunteers. Blood samples (5 ml) for assay of plasma concentrations of CNZ were obtained via the indwelling cannula immediately prior to dosing.

Table 2

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Precursor ion (Da)</th>
<th>Product ion (Da)</th>
<th>Dwell time (ms)</th>
<th>Fragmentation energy (V)</th>
<th>Collision energy (V)</th>
<th>Polarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sildenafil</td>
<td>475</td>
<td>100</td>
<td>200</td>
<td>120</td>
<td>30</td>
<td>Positive</td>
</tr>
<tr>
<td>Cinnarizine</td>
<td>369.2</td>
<td>167.1</td>
<td>200</td>
<td>100</td>
<td>10</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Fig. 1. In-vitro dissolution profiles of CNZ from the 16 formulae suggested by the factorial design. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
and at the time of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10.0, 12.0, and 24 h after oral administration. Samples were put into polyethylene heparinized tubes and were immediately centrifuged at 3000 rpm for 5 min. The plasma obtained was frozen at −20 °C in labeled tubes until LC-MS/MS analysis.

2.4.8.2. Sample preparation. Fifty μl of sildenafil, as internal standard (IS) (from a stock solution of concentration 3 μg/ml) and five mls of acetonitrile were added to each sample (0.5 ml plasma), vortexed for 1 min and centrifuged for 10 min at 4000 rpm (cooling centrifuge, Sigma, 2-16 PK). The organic layer was transferred to another tube filtered through 0.22 μm Millipore filter, then evaporated to dryness using vacuum concentrator (Eppendorf Vacufuge plus, Germany). Dry residues were reconstituted with 200 μl mobile phase (10 mM ammonium acetate pH 3.3: acetonitrile [35:65] (v/v)), and finally 3 μl was injected on the column for analysis.

Table 3
The composition of the prepared formulations in actual values (uncoded units) and their observed responses.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Responses a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y1</td>
</tr>
<tr>
<td>A  1</td>
<td>2 6 0 5</td>
</tr>
<tr>
<td></td>
<td>2 6 0 5</td>
</tr>
<tr>
<td></td>
<td>6 6 0 5</td>
</tr>
<tr>
<td>B  4</td>
<td>6 12 0 5</td>
</tr>
<tr>
<td></td>
<td>2 6 2 5</td>
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<td></td>
<td>6 6 2 5</td>
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<td></td>
<td>2 12 2 5</td>
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<tr>
<td></td>
<td>6 12 2 5</td>
</tr>
<tr>
<td>C  8</td>
<td>6 6 0 8</td>
</tr>
<tr>
<td></td>
<td>6 6 0 8</td>
</tr>
<tr>
<td></td>
<td>11 2 12 0 8</td>
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<td></td>
<td>12 6 12 0 8</td>
</tr>
<tr>
<td></td>
<td>13 2 6 2 8</td>
</tr>
<tr>
<td>D  14</td>
<td>6 6 2 8</td>
</tr>
<tr>
<td></td>
<td>15 2 12 2 8</td>
</tr>
<tr>
<td></td>
<td>16 6 12 2 8</td>
</tr>
</tbody>
</table>

a Mean ± standard deviation (n = 2).

Fig. 2. Normal plot for screening of the influences and the significances of the studied factors. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
2.4.8.3. LC-MS/MS assay of CNZ. Plasma concentrations of CNZ were analyzed using a validated LC-MS/MS method. LC system (Agilent 1260, Germany) coupled with triple quadrupole MS/MS detector (Agilent 6410, Germany) was used. The chromatographic separation was carried out on a C18 Poroshell 120, 4.6 x 50 mm 2.7 μm. The mobile phase was composed of 10 mM ammonium acetate pH 3.3: acetonitrile [35:65] (v/v). The flow rate was set as 0.4 ml/min. The analysis was operated at the MRM (multiple reaction monitoring) mode, and its MS parameters are shown in Table 2.

2.4.8.4. Pharmacokinetic analysis. After oral administration of the two treatments of CNZ; pharmacokinetic parameters were determined by plasma concentration time profiles by means of non-compartmental analysis using Kinetica® (version 5, Thermo Fischer Scientific). Maximum CNZ concentration ($C_{\text{max}}$, ng/ml), and time required to reach maximum CNZ concentration ($T_{\text{max}}$, h) were obtained from the individual plasma concentration time curves. The elimination rate constant ($K$, h⁻¹) was obtained from the slope of the linear regression of the log-transformed plasma concentration–time data in the elimination phase. The area under the plasma concentration–time curve (AUC, ng h/ml) was subdivided into AUC₀–₂₄ (was determined as the area under the plasma concentration–time curve up to the last measured sampling time and calculated by the linear trapezoidal rule), and area under the curve from zero to infinity (AUC₀–∞, ng h/ml); where $\text{AUC}_{0–\infty} = \text{AUC}_{0–24} + C_t/K$ ($C_t$ is the last measured sample concentration at time $t$).

![Surface Plot of Y1 (%) vs % Pluronic, Lipid : Drug](image1.png)

![Surface Plot of Y2 (%) vs % Pluronic, Lipid : Drug](image2.png)

![Surface Plot of Y3 (%) vs % Pluronic, Lipid : Drug](image3.png)

Fig. 3. Response surface plot showing the effect of different factors on the release profile of CNZ from the studied lipid beads. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
2.4.8.5. **Statistical analysis.** The optimized CNZ-lipid beads and the reference treatment (Stugeron®) were compared with respect to the relevant pharmacokinetic variables. The pharmacokinetic parameters $C_{\text{max}}$, AUC$_{0-24}$, and AUC$_{0-\infty}$ were compared between treatments statistically using ANOVA with a level of significance of 0.05 and a $P$-value smaller than 0.05 was considered significant.

3. Results and discussion

3.1. **Floating behavior**

All formulations exhibited immediate floating with no lag time in 0.1 N HCl 0.02% Tween 20 and floating was maintained up to 24 h, in contrast to most conventional floating systems (including gas-generating ones). This reflects the excellent floating ability of the prepared beads. The surface hydrophobicity imparted to the drug particles by the hydrophobic lipid coat, as well as the density of the investigated lipids (density of Gelucire 43/01, the major component, is 0.0856 g/cm$^3$) was responsible for this distinctive floating behavior. Similar results were reported by Shimpi et al. [13].

3.2. **Analysis of the factorial design**

The application of factorial design in pharmaceutical formulation development has played a key role in understanding the relationship between the independent variables and the responses to them. This helps the process of optimization by providing an empirical model equation for the response as a function of the different variables. The experiment runs with independent variables and the observed responses for the 16 formulations are shown in Fig. 1 and Table 3. It was observed that CNZ release profiles differed with changing the compositions of the prepared formulations. Some formulations showed initial burst release (reaching 68.87% in the first hour). Other formulations exhibited slow initial release. It is worthy to mention that the development of CNZ gastroretentive sustained release dosage form could be considered as a challenge because of the high solubility of CNZ in acidic media.

Fig. 2 represents normal probability plots of the effects of different formulation variables on the studied responses. The effects that lie along the normal probability line as round points are negligible (i.e. non-significant), whereas significant effects are those square points far from the normal probability line, i.e. not explained by natural experimental variation. The closer the point to the line the lower was its significance on the response. The plot can be divided into two regions. The region lies on the right (or positive) side of the line, where the factors and their interactions presented positive coefficients and the region on the left (or negative) side of the line, where the factors and their interaction presented negative coefficients [16].

The percent of drug released at different time intervals ($Y_1$, $Y_2$, and $Y_3$) (1, 5, and 8 h respectively) was synergistically affected by the percent of Pluronic F-127 (B). This might be attributed to its surface activity properties [17,18] resulting in improvement of CNZ solubility. Enhancement of drug release could be also due to the pore formation ability of the surfactant [19]. In contrast, lipid:drug-ratio (A) together with percent of Sterotex (C) had an antagonistic effect on CNZ release. Lipids have the ability to retard drug release as a result of the increase in the lipid matrix density and the subsequent increase in the diffusion path length through which the drug molecules have to transverse. Also, on increasing the ratio of Gelucire 43/01:Gelucire 50/13 (D), a negative effect was observed, this could be due to the higher lipophilic nature of Gelucire 43/01 (HLB = 1) compared to Gelucire 50/13 with its surface activity properties (HLB = 13). The relationship between the

![Fig. 4. Contour plot showing the effect of different factors on CNZ loading efficiency. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image)

Table 4

<table>
<thead>
<tr>
<th>Response</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_1$ (% drug released after 1 h)</td>
<td>$Y_1 = 44.45 - 6.10A + 4.13B - 8.57C + 0.90D + 0.38AB + 2.44AC + 1.31AD + 2.04BC + 0.11BD + 1.98CD + 1.27ABC + 1.56ABD + 0.94ACD + 1.28BCD + 0.086ABCD (R^2 = 0.8153)$</td>
</tr>
<tr>
<td>$Y_2$ (% drug released after 5 h)</td>
<td>$Y_2 = 69.62 - 7.99A + 6.21B - 8.03C - 1.64D + 1.92AB - 0.80AC + 0.52AD + 3.64BC - 0.63BD + 0.90CD + 2.94ABC + 1.11ABD + 0.064ACD - 0.58BCD - 1.40ABCD (R^2 = 0.9964)$</td>
</tr>
<tr>
<td>$Y_3$ (% drug released after 8 h)</td>
<td>$Y_3 = 79.80 - 5.97A + 6.20B - 8.03C - 3.11D + 2.40AB - 0.76AC + 0.46AD + 1.74BC + 0.72BD - 0.24CD + 0.61ABC - 0.050ABD - 0.74ACD - 0.089 BCD + 1.08ABCD (R^2 = 0.9709)$</td>
</tr>
<tr>
<td>$Y_4$ (% loading efficiency)</td>
<td>$Y_4 = 89.63 + 0.19A + 2.51B - 1.52C - 2.01D - 1.36AB + 4.41AC + 0.44AD - 1.49BC + 0.56BD + 0.83CD + 1.59ABC + 0.77ABD - 0.48ACD - 1.08BCD + 1.04ABCD (R^2 = 0.8401)$</td>
</tr>
</tbody>
</table>
dependent and independent variables was further elucidated using three dimensional surface plots as shown in Fig. 3. It could be concluded that the composition of the studied beads can be tailored in order to achieve the desired release profile of CNZ.

Regarding the loading efficiency, the percent of Pluronic F-127 (B) exhibited positive effect. Furthermore, it was clear that increasing Gelucire 43/01:Gelucire 50/13 ratio (D) increased the percent of drug entrapped. Finally, the percent of Sterotex (C) was observed to have a negative effect on the percent of drug loaded into the beads. The relationship between the dependent and independent variables was further elucidated using two dimensional contour plots as shown in Fig. 4.

3.3. Optimization

The linear regression models of the studied responses in terms of coded factors are represented in Table 4. The equations were left in their complete form without omitting any terms, to avoid loss of any important information [20]. After generating the polynomial equations relating the dependent and independent variables, the optimum formula was automatically generated by the software based on the desirability function aiming to attain zero order release profile of CNZ over 12 h with initial target release of 17% in the first hour to act as loading dose as well as the highest loading efficiency. The constraints used in the optimization process were listed in Table 1.

As shown in Table 5, for the suggested formula, the optimized values for the lipid:drug ratio (A), the percent of Pluronic F-127 (B), the percent of Sterotex (C), and the ratio of Gelucire 43/01:Gelucire 50/13 (D) were 5.23:1, 6%, 2%, and 5:1 respectively. The composite desirability for the optimized formula was 0.45.

To examine the validity of the optimization process, a new batch of the optimized formula according to the computer levels was prepared and evaluated. As shown in Table 5, a small residual between the observed and expected values of the studied responses was obtained. Fig. 5 demonstrates that the optimized formulation exhibited a release profile which was close to that of the suggested target release profile. Accordingly, these results validate the reliability of the optimization procedure.

3.4. Morphological examination and scanning electron microscope (SEM)

As shown in Fig. 6, the optimized formula beads were spherical, showing a smooth surface. SEM micrographs of CNZ-lipid beads are shown in Fig. 7 at different magnification powers before and after drug release. Initially before release, the beads were nearly spherical, with quite smooth surface without any pores or cracks (Fig. 7a, c and e). After eight hours of release study in 0.1 N HCl, cracks (Fig. 7b) and pores (Fig. 6d and f) were observed on the surface of the beads (indicated by red arrows). This could be due to dissolution of Pluronic F-127 (the only soluble excipient) and this might possibly explain the observed positive effect of Pluronic F-127 on CNZ release mentioned earlier [19].

3.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) provides information about melting, crystallization, decomposition and physicochemical status of the drug as well as its interaction with different excipients [21]. As shown in Fig. 8, pure CNZ exhibited a single sharp endothermic peak at 121 °C corresponding to its melting point [22]. Sterotex showed a single sharp endothermic peak at 60 °C and Pluronic F-127 showed sharp endothermic peak at 55.5 °C, corresponding to their melting points. For Gelucire 43/01 and Gelucire 50/13, each showed single endothermic peak at 39.7 °C and 41.6 °C respectively corresponding to their melting points.

The endothermic peak of the drug was shifted to a lower temperature in the optimized formula and in all drug-excipient mixtures (1:1 w/w) except in case of Gelucire 50/13, where the peak of the drug was not further observed. In case of Gelucire 50/13, the complete disappearance of the endothermic peak corresponding to the melting CNZ, might be due to CNZ complete solubility in the fused Gelucire 50/13 before reaching its melting point during the DSC scan. In the other thermograms, the presence of CNZ shifted endothermic peak, indicates the partial solubility of

![Fig. 5](image-url) Release profile of CNZ from the optimized formula compared to target release profile. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

![Fig. 6](image-url) Light photograph for the optimized formula. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
CNZ in the melted excipients and that some CNZ crystals still exist upon reaching the melting point of CNZ in the DSC scan [23]. It was worthy to note that the difference in thermal behavior of CNZ observed with both Gelucire 50/13 and Gelucire 43/01 might be due to the different chemical structure of the studied Gelucires where, Gelucire 43/01 is a hard fat and Gelucire 50/13 is Stearoyl macrogol-32 glycerides.

3.6. X-ray diffraction (XRD)

As shown in Fig. 9, pure CNZ is highly crystalline as indicated by the numerous sharp characteristic peaks at angles of 2θ at 10.29, 20.79, 20.955, and 24.56. The existence of these peaks in diffractograms of the studied physical mixtures with the excipients and the optimized formula indicates that CNZ maintained its crystalline state during mixing with the studied excipients and preparation of the optimized formula.

3.7. In-vivo study

Mean plasma concentrations of CNZ-time profile achieved after single administration of both the optimized CNZ-lipid beads equivalent to 25 mg CNZ and Stugeron® 25 mg are shown in Fig. 10. The mean pharmacokinetic parameters were shown for both formulae in Table 6. Statistical analysis showed that there were significant differences (p < 0.05) between the values of $T_{\text{max}}$, AUC$_{0-24}$, and AUC$_{0-\infty}$ of the optimized CNZ-loaded lipid beads compared to Stugeron® tablets while statistical insignificant difference (p > 0.05) was observed in case of $C_{\text{max}}$.
The mean AUC_{0–24} and the AUC_{0–∞} of the optimized CNZ-loaded lipid beads were found to be 4.23 times, and 6.04 times that of Stugeron^® tablets respectively. The delayed T_{\text{max}} increased C_{\text{max}} and higher AUC indicate a slow and prolonged absorption of CNZ from the optimized CNZ loaded lipid beads formula with higher bioavailability and more extended plasma concentration–time profile over 24 h in comparison with the commercially available Stugeron^® tablets. This could be due to the increased gastric residence of CNZ beads as well as the gradual release of CNZ in its acidic medium. Also, it could be suggested that the incorporation of Pluronic F-127 and Gelucire 50/13, which are reported for the bioavailability enhancing effect [24,25], contributed to the improvement of CNZ bioavailability.

Fig. 8. DSC thermograms of CNZ, Sterotex NF, Pluronic F-127, Gelucire 43/01, Gelucire 50/13, physical mixtures (1:1), and the optimized formula.
4. Conclusions

Promising, novel as well as simple optimized floating gastro retentive CNZ lipid beads were successfully developed. The beads were characterized by: excellent floating behavior, zero order release profile for 12 h, high entrapment efficiency, and potentially suitable for once daily administration. The suggested floating lipid beads could be easily tailored by changing their composition to achieve the desired drug release profile, thereby considered an effective approach in developing extended release formulations for poorly soluble basic drugs with improved bioavailability.

Conflict of interest

The authors confirm that there is no conflict of interest.

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References


