ABSTRACT

Objective: The present study aims to utilize the nanotechnology technique to formulate the Cinnarizine (CNZ) in the form of solid self-nanoemulsifying system to enhance the dissolution and hence the bioavailability.

Methods: Screening study for solubility of CNZ in different vehicles was carried out. The selected system was optimized for saturated solubility, globule size, zeta potential, polydispersity index (PDI) and self-emulsification time. The solidified nanoemulsion was prepared using Aeroperl 300, Aerosil 200, hydrophilic nanosilica and Neusilin US 2 as porous carrier materials. The compressed CNZ tablets were evaluated regarding their physicochemical characteristics, in-vitro release, and bioavailability study.

Results: Self nano-emulsifying system composed of Labrafil (oil), tween 80 (surfactant), and transcutol (cosurfactant) was successfully developed with a droplet size range of 11.37-92.58 nm. The in-vitro release results revealed that the developed formulation improved the release of CNZ and enhanced the bioavailability in the rabbits (190%) more than the commercial product (Stugeron® tablets).

Conclusion: Solid self-nano-emulsifying system of CNZ was successfully developed by different ratios of Labrafil (oil), tween 80 (surfactant), transcutol (cosurfactant) and solidified by the adsorption on hydrophilic nano silica and the optimized formula could be expected to increase and improve the bioavailability of CNZ.

Keywords: Pseudo-ternary phase diagram, Self-nanoemulsion, Porous carriers, Bioavailability study.

INTRODUCTION

Microemulsions are clear thermodynamically stable, composed of an isotropic mixture of natural or synthetic oil with lipophilic or hydrophilic surfactant and co-solvents [1-3]. They can act as drug delivery vehicles by incorporating a wide range of drug molecules [4, 5]. Self-nanoemulsifying drug delivery systems undergo emulsification by gentle agitation in the presence of gastric fluid to form oil in water emulsion with droplet size ranging between 10-200 nm as self-nanoemulsifying drug delivery system whereas droplet size ranging between 100 and 200 nm are known as selfmicro emulsifying drug delivery system [67]. Self-nanoemulsifying drug delivery system have many advantages include; increased drug loading, enhancing of drug penetration through the biological membranes so increased the bioavailability, the ease of preparation due to the spontaneous formation, thermodynamic stability, transparent and elegant appearance [8-9]. There are few limitations associated with self-nanoemulsion (SNE) when presented in capsules, including the precipitation of the drug during manufacture and storage and the incompatibility of the formulation components with the capsule shell [10]. To overcome these problems, the concept of solid SNE was developed. Various techniques, such as freeze drying, spray drying, and adsorption on porous carriers, can be employed to convert liquid SNE into solid depending [11, 12].

Cinnarizine (CNZ) is a piperazine derivative with high affinity to H1 receptors that can be used in allergic conditions. However it is practically insoluble in water (aqueous solubility less than 1 μg/ml exhibit low oral bioavailability, which due to its poor and pH-dependent dissolution of the drug rate [13-15]. The objective of this work was to utilize nanotechnology to formulate CNZ into SNE tablets that increase drug dissolution and in turn, increasing the bioavailability of the drug.

MATERIALS AND METHODS

Materials

Cinnarizine was kindly gifted by Arab drug company (ADCO), Egypt. Tween®80 (Polyoxyethylene sorbitan monooleate, HLB=15), Cremophore RH 40 (Polyoxyethylene 40 hydrogenated castor oil, HLB 14-16) and Aeroperl 300 (granulated silicon dioxide) were kindly gifted from Degussa, Germany. Avicel® PH 102 (Microcrystalline cellulose), Ac-Di-Sol® (crosscarmellose sodium) and Propylene glycol were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Labrafri M 1944 CS (Oleoyl macrogol-glycerides) and Labrafac lipophile WL 1349 (Medium chain triglycerides) were kindly gifted by Gattefosse, France. Soyabean oil and oleic acid (Octodecenoic acid) were purchased from Lab Chemicals Trading Co., Egypt. Hydrophilic nano silica and Neusilin US 2 (Magnesium Aluminometasilicate) were purchased from NanoTech Co., Egypt. Transcutol HP was a gift from Gattefossé@ France.

Methods

Solubility studies

An excess amount of CNZ was added to 2 ml of various oils, surfactants, and cosurfactants, and mixed by a vortex mixer. The mixture was kept at 25 °C for 72 h till equilibrium. The sample was centrifuged at 1000 rpm for 10 min and filtered. The filtrate diluted with methanol and then the amount of CNZ was determined by UV spectroscopy at λmax= 254.

Construction of pseudo-ternary phase diagrams

The selected oil (Labrafri), surfactant (tween 80) and co-surfactant (transcutal) were used to prepare the self-nanoemulsion preconcentrate system. 36 possible ratios were used to construct the phase diagram. 0.1 ml of each of the prepared self-nanoemulsion preconcentrates were mixed using a vortex mixer then diluted with 100 ml deionized water, and magnetically stirred at 500 rpm for 2 min at room temperature. The diluted products were inspected against a dark background and were discriminated as clear, turbid or biphasic [16]. Only the clear emulsions are considered as nanoemulsion with droplet size 200 nm or less [17].
Optimization of the selected system

Saturated solubility study

Excess amount of the drug was added into 2 ml of each of the clear ratios in the selected system (table 1) and mixed by a vortex mixer and moved to thermodynamically water bath shaker for 72 h at (25±0.5 °C) then centrifuged at 5000 rpm for 20 min. The supernatant was diluted with phosphate buffer pH 6.8 using magnetic stirrer at 500 rpm for 2 min [18]. The amount of dissolved drug was determined using UV spectroscopy at λmax 254 nm.

Table 1: The composition of the different self-nanoemulsion pre-concentrates systems

<table>
<thead>
<tr>
<th>System</th>
<th>Labrafil M 1944 CS</th>
<th>Tween 80</th>
<th>Transcutol</th>
</tr>
</thead>
<tbody>
<tr>
<td>System I</td>
<td>20%</td>
<td>50%</td>
<td>30%</td>
</tr>
<tr>
<td>System II</td>
<td>20%</td>
<td>60%</td>
<td>20%</td>
</tr>
<tr>
<td>System III</td>
<td>20%</td>
<td>70%</td>
<td>10%</td>
</tr>
<tr>
<td>System IV</td>
<td>10%</td>
<td>50%</td>
<td>40%</td>
</tr>
<tr>
<td>System V</td>
<td>10%</td>
<td>60%</td>
<td>30%</td>
</tr>
<tr>
<td>System VI</td>
<td>10%</td>
<td>70%</td>
<td>20%</td>
</tr>
<tr>
<td>System VII</td>
<td>10%</td>
<td>80%</td>
<td>10%</td>
</tr>
</tbody>
</table>

*In each system 0.1 ml pre-concentrate was diluted with 100 ml deionized water.

Robustness to dilution of SNE

Robustness to dilution was studied by diluting the prepared SNE (2 ml) with (200 ml) water. The diluted SNE were stored for 12 h and observed visually for clarity, signs of phase separation, and the quality of emulsion produced [19].

Particles size analysis and zeta potential measurement of SNE

The prepared SNE system were diluted with water, and the average particles size were determined using dynamic light scattering by Malvern Zetasizer 3000, Malvern Ltd., UK [20]. Zeta potential was determined using a photon correlation spectrometer based on the laser light scattering phenomenon. Samples were diluted 200 times with deionized water prior to measurement [21].

Self-emulsification time and percentage of transmittance

Determination of self-emulsification time was carried out by dilution of 0.5 ml of SNE in a glass beaker with 100 ml distilled water then mixed gently by using magnetic stirrer at room temperature with a rotation speed of 100 rpm. The time is taken by the SNE to form homogenous nano emulsion was measured, and the percentage of transmittance was measured at 638.2 nm, using distilled water as the blank by UV spectrophotometer [22].

Development of CNZ liquid-SNE

CNZ (25 mg) was added to the optimized SNE (0.1 ml) in a glass vial then mixed by Sonicator for 20 min till drug was dissolved. The mixture was stored at room temperature until further investigations.

Surface morphology determination of the optimized CNZ-SNE

The high-resolution transmission electron microscope (TEM, JEM-1400, Japan) was used to evaluate the morphology of the liquid CNZ-SNE. The samples were diluted up to 100 times with deionized water to form nano emulsion. In the TEM technique, a drop of the formed nano emulsion was loaded on a carbon-coated copper grid. The excess was removed immediately using filter paper. 2% w/v aqueous solution of phosphotungstic acid (staining agent) was directly added to the grid and left for 45 seconds. Then, they were allowed to dry for 1 h. After drying, the grid was directly investigated and photographed using (TEM, JEM-1400, Japan).

Development of CNZ solid-self nano emulsifying system

The optimized liquid SNE formulation was transformed into solid granules through using different inert porous carriers (Aeroperl 300, Aerosil 200, hydrophilic nano silica and Neusilin US2) as adsorbing agents due to their oil adsorption property [23]. The porous carriers were added to a fixed volume of the liquid SNE (0.1 ml) containing (25 mg CNZ) in increments amounts with shaking until the formation of free fluid powder. The final weight of the formed free fluid granules were determined (table 2).

Preparation of CNZ self nano emulsifying tablets

The resulting granules were mixed with the binder (Avicel PH101) and 3% w/w of disintegrant (Ac-Di-Sol) was added to the mixture and mixed for 10 min and complete the final weight with lactose (table 2). Prepared powders were compressed into an oval, curve faced tablets of 500 mg using tablet press machine (Rimex Mini Press, Model RSB-4, Kanavati Engineering).

Optimization of the prepared CNZ self-nano emulsifying tablets

Physicochemical properties of the prepared CNZ-SNE tablets

The weight uniformity was determined for the tablets, and the friability was also determined using a friabilator at 25 rpm for 4 min. The friability is expressed in percent of weight loss. The uniformity of CNZ content was determined by crushing tablets from each system and determining the content by dilution with methanol and measuring spectrophotometry for their CNZ content. The mean hardness was measured using Erweka hardness tester and expressed in kg. Finally, the disintegration time of tablets was determined in minutes using the disintegration test apparatus (Pharma Test, Type PFT23).

In vitro release of CNZ from the prepared CNZ-SNE tablets

The dissolution of CNZ from the prepared tablets was performed in 900 ml of standard phosphate buffer (pH 6.8) at 37±0.5 °C using dissolution tester (Erweka, model, Germany). Apparatus II (rotating paddle the USP Dissolution test), at a rotation of 75 rpm. 10 ml from the dissolution medium was withdrawn at 5, 10, 15, 20, 30, 45 and 60 min. Samples were filtered through 0.45-μm filter (PVDF membrane, Millipore Corp., USA) and analyzed spectrophotometry (Shimadzu 1800, Japan) at 254 nm. The drug release from the formulation was measured and compared with the conventional marketed CNZ tablet (Stugeron®).

Table 2: Preparation of self-nano emulsifying tablets

<table>
<thead>
<tr>
<th>Tablet components</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeroperl 300-SNE</td>
<td>290 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerosil 200-SNE</td>
<td></td>
<td>340 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrophilic nanosilica-SNE</td>
<td></td>
<td></td>
<td>230 mg</td>
<td></td>
</tr>
<tr>
<td>Neusilin US2-SNE</td>
<td></td>
<td></td>
<td></td>
<td>256 mg</td>
</tr>
<tr>
<td>Tablet components</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% Avicel PH101 (Binder)</td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
<td>F4</td>
</tr>
<tr>
<td>20% Ac-di-sol (disintegrant)</td>
<td>100 mg</td>
<td>100 mg</td>
<td>100 mg</td>
<td>100 mg</td>
</tr>
<tr>
<td>3% Ac-di-sol (disintegrant)</td>
<td>15 mg</td>
<td>15 mg</td>
<td>15 mg</td>
<td>15 mg</td>
</tr>
</tbody>
</table>

* 25 mg of the drug was added to each formulation, *The weight of each prepared tablet was completed to 500 mg by addition of lactose as filler
Bioavailability

Study design

The in-vivo study was conducted according to the institutional guidelines of the Animal Ethics Committee of the faculty of pharmacy Beni-suief University. The study was carried out using 12 healthy albino rabbits (New Zealand) weighing 2.5-3.0 kg to compare the CNZ pharmacokinetics (treatment A) of the optimized SNE-tablet to the commercially tablet Stugeron® (treatment B). The animals were fasted for 24 h before the administration of dose (allow to drink water) and were divided into two equal groups. The first group was administered the reference (Stugeron tablet) at a dose of 25 mg/kg while the second group was administered CNZ-SNE tablet at a dose of 25 mg/kg [24].

Sample collection

Blood samples (2 ml) were collected into heparinized tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 10, 12 and 24 h after the administration. The blood samples were centrifuged at 4,000 rpm and the separated plasma samples were stored at −20 °C in a deep freezer, until further analysis.

Extraction procedure

0.5 ml from plasma was added to 0.5 ml (2 µg/ml) of internal standard (Meclozine HCl) and acidified by addition of 0.2 ml of HCl (1N), then 5 ml of chloroform was added and centrifuged for about 10 min at 2,000 rpm. The chloroformic layer was separated and evaporated till dryness. Finally, the residual was reconstituted with 500 µl of the mobile phase, vortex then filtered through filter disk in eppendorf tube, then analyzed by HPLC.

Chromatographic conditions

A reported validated and sensitive HPLC (Bond pack C18 column) of modified Hasan Extraction et al., method was used [25]. The mobile phase consists of acetonitrile: water (6:4) in which 1.5 gram of sodium heptano-sulphonate was dissolved per liter. The pH of the mobile phase was adjusted to 3.5 with 0.1N sulphuric acid. After filtration through a membrane filter, the mobile phase was degassed and pumped at flow rate 1 ml/min. The UV detector was adjusted at 254 nm and the element peaks were investigated using peak height ratio, all assays were performed at ambient condition.

Determination of pharmacokinetic parameters

The Cmax, AUC0-∞, t1/2 and MRT (mean resident time) were calculated using the plasma concentration-time curve in the WinNonlin Nonlinear compartment Program. One-way analysis of variance was employed to assess the significance of the difference.

RESULTS AND DISCUSSION

Optimization of the selected systems

Robustness to dilution

The dilution capacity of each system was determined to study the effect of dilution on the stability of the nano emulsion when subjected to dilution by the GIT fluids; this may result in a gradual desorption of surfactant located at the droplet interface. This process depends upon the requirement of the surfactant for maintaining the aqueous phase concentration equivalent to its critical micelle concentration [26]. The seven selected SNE systems (table 1) are those, which, upon dilution with distilled water produced a clear nano emulsion when observed visually.

Globule size and poly dispersity index (PDI)

The droplet size of the emulsion is a critical factor in self-emulsification performance because it determines the rate and extent of drug release and consequently its absorption [27]. All the prepared SNE had droplets size in the range of 11.37-92.58 nm (table 3). There is a relationship between the droplet size and the concentration of the surfactant being used. It has been reported that droplets with smaller mean droplet size could be obtained upon increasing the surfactant concentration [27]. Similar in this study, it was noted that upon increasing the surfactant ratio the obtained droplets were smaller in size. This could be explained by the fact that the surfactant stabilizes the O/W interface, and its concentration increased at the interface upon decreasing the oily content. That attributed to a decrease in the emulsion particle size thus a more clear-appearing emulsion was produced [28]. However, an increase in co-surfactant concentration was accompanied by an increase in droplet size. This could be attributed to the interfacial disruption elicited by enhanced water penetration into the oily droplets mediated by the increased co-surfactant concentration and leading to the ejection of oil droplets into the aqueous phase [29].

PDI is the ratio between the standard deviation and the mean globule size; it indicates the uniformity of the globule size within the system. The lower the value of PDI, the higher uniformity of the globule size [30]. It was found that PDI range between 0.098-0.353 indicating a good uniformity of globule size.
Zeta potential measurements

The zeta potential was studied to indicate the stability and the degree of repulsion between the individual particles. The presence of a small negative charge of zeta potential (table 3) has no effect on the stability of nano-emulsion.

Development of CNZ-liquid SNE

From the previous physicochemical character, System VI containing 10% Labrafil oil, 70% tween 80 and 20% transcutal was selected for the further characterizations.

Surface morphology determination of the liquid CNZ-SNE

Transmission electron microscopy for the clear CNZ nano-emulsion was study, and the nano-emulsion droplets were observed as regular dark droplets surrounded by a thick bright frame well-identified spherical shaped droplets (fig. 3). The emulsion droplets generated have a smooth surface with no crystals of the drug on the surface.

Development and optimization solid CNZ-SNE granules

The simplest technique to convert liquid SNE to solid SNE granules is by adsorption on porous carriers. The different formulation composition of CNZ self-nano-emulsifying granules showed good flow ability with regard to Carr’s index, in the range of 14.77 to 17.93, Hausner’s ratio less than 1.2 and angle of repose less than 31° as shown in (table 4).

Evaluation of CNZ self-nano emulsifying tablets

Table 5 shows the physicochemical properties of the prepared tablets, the drug content were found to be in the range of (96.04±4.3692-101.56±4.75%) indicating proper mixing and good flow ability and uniformity of drug content of the formulation. The friability values were in accepted range according to the criteria of the USP (less than 1%). The recorded hardness values were 3.97±0.3 kg to 4.98±0.21 kg. The disintegration time were less than two minutes reflecting the efficiency of Ac-di–sol (3%) as a disintegrant.

Table 3: Optimization of the selected self-nano emulsifying systems

<table>
<thead>
<tr>
<th>System</th>
<th>Solubility</th>
<th>Z-Average (nm)</th>
<th>PDI*</th>
<th>Zeta potential (MV)</th>
<th>Emulsification time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>System I</td>
<td>52.58±4.56</td>
<td>92.58±4.56</td>
<td>0.353</td>
<td>−9.73±0.52</td>
<td>61</td>
</tr>
<tr>
<td>System II</td>
<td>63.13±5.34</td>
<td>71.47±2.34</td>
<td>0.286</td>
<td>−10.55±0.57</td>
<td>74</td>
</tr>
<tr>
<td>System III</td>
<td>71.72±4.392</td>
<td>41.72±3.13</td>
<td>0.227</td>
<td>−12.43±0.49</td>
<td>42</td>
</tr>
<tr>
<td>System IV</td>
<td>90.58±3.803</td>
<td>18.59±0.1</td>
<td>0.127</td>
<td>−13.76±0.36</td>
<td>66</td>
</tr>
<tr>
<td>System V</td>
<td>84.89±5.373</td>
<td>13.89±0.07</td>
<td>0.239</td>
<td>−9.93±0.12</td>
<td>42</td>
</tr>
<tr>
<td>System VI</td>
<td>114.81±6.193</td>
<td>11.03±0.05</td>
<td>0.0973</td>
<td>−9.78±0.33</td>
<td>23</td>
</tr>
<tr>
<td>System VII</td>
<td>101.27±6.51</td>
<td>14.37±0.05</td>
<td>0.0941</td>
<td>−9.13±0.22</td>
<td>20</td>
</tr>
</tbody>
</table>

*PDI: Poly dispersity Index, Data expressed as mean±standard deviation

Fig. 3: Transmission electron microscopy of the optimized CNZ liquid-SNE with different magnification

Table 4: Micrometrics properties of the prepared granules

<table>
<thead>
<tr>
<th>Formula</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carr’s index</td>
<td>16.40</td>
<td>17.93</td>
<td>14.77</td>
<td>16.33</td>
</tr>
<tr>
<td>Hausner’s ratio</td>
<td>1.196</td>
<td>1.218</td>
<td>1.173</td>
<td>1.195</td>
</tr>
<tr>
<td>Angle of repose±S. D*</td>
<td>28.42±0.48</td>
<td>30.32±0.49</td>
<td>27.36±0.28</td>
<td>29.28±1.03</td>
</tr>
</tbody>
</table>

*Standard deviation

Table 5: Physicochemical Properties of the prepared tablets

<table>
<thead>
<tr>
<th>Formula</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug content (%)±S. D*</td>
<td>96.04±4.36</td>
<td>101.56±4.75</td>
<td>98.25±3.92</td>
<td>99.69±2.95</td>
</tr>
<tr>
<td>Hardness (kg)±S. D*</td>
<td>4.74±0.45</td>
<td>3.97±0.33</td>
<td>4.98±0.21</td>
<td>4.82±0.51</td>
</tr>
<tr>
<td>% Friability</td>
<td>0.78</td>
<td>0.83</td>
<td>0.58</td>
<td>0.68</td>
</tr>
<tr>
<td>Disintegration time (Seconds)±SD*</td>
<td>110.45±6.73</td>
<td>98.11±7.23</td>
<td>63.38±4.12</td>
<td>86.45±7.45</td>
</tr>
</tbody>
</table>

*Standard deviation, *All the physicochemical properties were in the accepted range.
The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

REFERENCES


