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To link to this article: https://doi.org/10.1080/10837450.2017.1335321
RESEARCH ARTICLE

Zero-order release and bioavailability enhancement of poorly water soluble Vinpocetine from self-nanoemulsifying osmotic pump tablet

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ABSTRACT

Solid self-nanoemulsifying (S-SNEDDS) asymmetrically coated osmotic tablets of the poorly water-soluble drug Vinpocetine (VNP) were designed. The aim was to control the release of VNP by the osmotic technology taking advantage of the solubility and bioavailability-enhancing capacity of S-SNEDDS. Liquid SNEDDS loaded with 2.5 mg VNP composed of Maisine<sup>TM</sup> 35-1, Transcutol<sup>®</sup> HP, and Cremophor<sup>®</sup> EL was adsorbed on the solid carrier Aerosper<sup>®</sup>. S-SNEDDS was mixed with the osmotic tablet excipients (sodium chloride, Avicel<sup>®</sup>, HPMC-K4M, PVP-K30, and Lubriderm<sup>®</sup>), then directly compressed to form the core tablet. The tablets were dip coated and mechanically drilled. A 3<sup>2</sup>x<sup>2</sup> full factorial design was adopted. The independent variables were: type of coating material (X<sub>1</sub>), concentration of coating solution (X<sub>2</sub>), and number of drills (X<sub>3</sub>). The dependent variables included % release at 2 h (Y<sub>1</sub>), at 4 h (Y<sub>2</sub>), and at 8 h (Y<sub>3</sub>). The in vivo performance of the optimum formula was assessed in rabbits. Zero-order VNP release was obtained by the single drilled 1.5% Opadry<sup>®</sup> CA coated osmotic tablets and twofold increase in VNP bioavailability was achieved. The combination of SNEDDS and osmotic pump tablet system was successful in enhancing the solubility and absorption of VNP as well as controlling its release.

1. Introduction

VNP is a semi-synthetic drug derived from the alkaloid vincamine obtained from the leaves of the lesser periwinkle plant <i>Vinca minor</i> (Jha et al. 2012). It is used for patients suffering from neurodegenerative conditions, such as Parkinson’s and Alzheimer’s disease owing to its anti-inflammatory and cognition improvement properties (Jeon et al. 2010). Unfortunately, VNP usefulness is limited owing to its poor bioavailability (7%) resulting from both its low aqueous solubility (5 μg/mL) and extensive hepatic first-pass metabolism (Mao et al. 2013). This leads to low VNP concentration in the brain and limited clinical response. VNP is a weak basic drug whose solubility is pH dependent with higher solubility in acidic media (Nie et al. 2011). It shows biphasic elimination with alpha half-life (t<sub>1/2</sub>) of 0.136 h and beta t<sub>1/2</sub> of 4.83 h (Vereczkey et al. 1979). As a result of its short t<sub>1/2</sub> frequent dosing (three times daily) is required, which is inconvenient for patients with dementia and results in poor compliance (El-Laithy et al. 2011).

Since the bioavailability of VNP is limited in part by its solubility, several drug delivery systems with improved VNP solubility have been developed including cyclodextrin inclusion complexes (Aburahma et al. 2010; Ding et al. 2015), solid dispersions (Chen et al. 2009; Kharsoum et al. 2013), nanoparticles (Li et al. 2014; Wang & Xu 2016), solid lipid nanoparticles (Luo et al. 2006; Morsi et al. 2013), nanostructured lipid carriers (Lin et al. 2014), mixed polymeric micelles (El-Dahmy et al. 2014), and self-microemulsifying systems (Chen et al. 2008). Also, new salt forms of VNP (perchlorate, phosphate, and citrate) have been successfully formed and showed higher solubility when compared to VNP (Hasa et al. 2011; Ma et al. 2016).

Another approach to improve VNP solubility is the preparation of solid self-nanoemulsifying drug delivery systems (S-SNEDDS). Nanodized systems help in improving the solubility and dissolution rate and hence the bioavailability of poorly water soluble drugs. This is attributed to the increased surface area available for dissolution as described by the Noyes–Whitey (Noyes & Whitney 1897) and Ostwald–Freundlich equations (Schekin & Rusanov 2008). SNEDDS are lipid-based systems used for enhancing the solubility and bioavailability of hydrophobic drugs. Another advantage of SNEDDS is that both long and medium chain fatty acids used in their preparation are absorbed into the lymphatic system through Peyer’s patches (Chudasama et al. 2014). Thus, VNP avoids passing through the portal circulation and first-pass metabolism. This can further help in enhancing VNP bioavailability. When these liquid excipients are loaded on solid carriers, S-SNEDDS is formed, which is characterized by high stability and can be formulated in different dosage forms (Oh et al. 2011). S-SNEDDS produce oil-in-water nanoemulsions after oral administration by the GI motility of the stomach and intestine (Constantiniades 1995).

Another limitation of VNP is its short t<sub>1/2</sub>. This makes it a good candidate for the preparation of controlled release dosage forms. Several approaches have been explored to formulate S-SNEDDS into controlled release systems namely: polymer coated and matrix pellets (Serratoni et al. 2007; Zhang et al. 2012; Miao et al.
2014), microspheres (You et al. 2006; Yi et al. 2008), microcapsules (Zvonar et al. 2010, 2012), nanocapsules (Zvonar et al. 2012; Park et al. 2013), molded tablets (Patil et al. 2009), matrix tablets (Nazzal & Khan 2006; Wang et al. 2011), and osmotic pump tablets (Wei et al. 2007; Zhang et al. 2013). Osmotic systems are characterized by zero-order release kinetics unaffected by pH changes, which is a very beneficial feature for pH sensitive drugs like VNP.

Herein, we discuss the formulation of VNP solid self-nanoemulsifying osmotic pump tablet (VNP-SNEOPT). The formulation of SNEDDS will help in dissolving VNP and will increase the rate of drug absorption. Also, the dispersion of VNP in the oil globules will help in evading first-pass metabolism by enhancing the absorption through the lymphatic system. This can improve the bioavailability of VNP. Formulating SNEDDS into solid S-SNEDDS and then into SNEOPT will control the release rate and make the plasma concentrations more stable (Barzegar-Jalali et al. 2008).

A powder has very limited ability to retain liquids while maintaining plasma concentrations more stable (Barzegar-Jalali et al. 2008). Bioavailability of VNP. Formulating SNEDDS into solid S-SNEDDS and then into SNEOPT will control the release rate and make the plasma concentrations more stable (Barzegar-Jalali et al. 2008). Hence, it is very challenging to formulate a successful tablet formulation from the liquid S-SNEDDS. In this work, we describe the preparation, evaluation, and optimization of VNP-SNEOPT. We also investigate the effect of changing OPT coating parameters on the in vitro release and the in vivo pharmacokinetic performance of VNP from SNEOPT.

2. Materials and methods

2.1. Materials

Vinpocetine (VNP), manufactured by Covex (Madrid, Spain), was kindly supplied by Amriya Pharmaceutical Industries (Alexandria, Egypt). Cremophor EL and polyvinylpyrrolidone (PVP-K30) were kind gifts from BASF (Ludwigshafen, Germany). Transcutol HP and MaisineTM 35-1 were kind gifts from Galtefosse (Lyon, France). Aeroperl® 300 Pharma was supplied by Evonik Industries AG (Rheinfelden, Germany). Cellulose acetate (CA) was provided by Prolabo (Paris, France). Opadry® CA (a mixture of CA and polyethylene glycol 3350 in a ratio of 28:72) and hydroxypropyl methyl cellulose K4M (HPMC-K4M) were kind gifts from Colorcon (Indianapolis, IN). Polyethylene glycol 4000 (PEG) was supplied by Winlab Ltd. (Leicestershire, UK). Microcrystalline cellulose (Avicel® PH101) was purchased from FMC Corporation (Philadelphia, PA). Lubrispharm® was a kind gift from SPI Pharma (Septemberes-Vallons, France). Sodium chloride was purchased from El-Gomhouria Co. (Cairo, Egypt). Hydrochloric acid, glycerol and acetone were obtained from El-Nasr Pharmaceutical Chemicals Co. (Qaliubiya, Egypt). All other chemicals used were of pharmaceutical grade.

2.2. Preparation of the asymmetrically coated VNP osmotic tablets using the dip-coating technique

The S-SNEDDS and core tablet were prepared according to our previous work (El-Zahaby et al. 2016). Briefly, SNEDDS was composed of 25 mg MaisineTM 35–1, 125 mg Cremophor EL and 100 mg Transcutol® HP loaded with 2.5 mg VNP. The SNEDDS was loaded on 200 mg Aeroperl® by simple mixing using mortar and pestle to form the S-SNEDDS. Avicel® (133.3 mg), HPMC-K4M (14.2 mg), sodium chloride as osmotic agent (266.7 mg), PVP-K30 (28.5 mg), and Lubrispharm® (4.5 mg) were then mixed with the S-SNEDDS to form the core tablet with total weight of 900 mg.

The osmotic tablets were then asymmetrically coated using the dip-coating technique. In a preliminary study, the tablets were coated using three different coating solutions dissolved in acetone containing 10% of the non-solvent glycerol. The coating materials used were CA (2.5%, 5%, or 10%), Opadry® CA (2.5% or 5%), or a mixture of CA and PEG (80:20) similar to Opadry® CA. The tablets were coated to a weight gain of 10%. In another preliminary experiment, the core tablets were coated using 2.5%, 5%, or 7.5% Opadry® CA solutions by fixing the number of dippings to 2 × 10 s dippings All tablets were air-dried for 5 s and then immersed in a water quench bath for 3 min. The tablets were then air-dried at room temperature (25°C) for 12 h. A small orifice was drilled through the coated tablet using a standard 800 μm diameter mechanical micro-drill (dental bur FG 330, Midwest Dental, Wichita Falls, TX). The coated tablets had either one drill on one side or two drills with one on each side. The coat was examined under projection microscope (PRM-18T, Radical® Instruments, Haryana, India) before and after immersion into the release media to check the membrane porosity. Briefly, the coating membrane was removed from the tablet core, fixed on a slide using a glass cover and examined under the microscope.

2.3. Statistical design of the study

A 3² × 2¹ full factorial design was employed to compare the formulation and process variables. Generation and analysis of the experimental design were carried out using SPSS 17.0 software (SPSS Inc., Chicago, IL). The independent variables selected were the type of coating material (X₁): CA, CA with PEG (80:20), and Opadry® CA, concentration of the coating solution (X₂): 1%, 1.5%, and 2%, and the number of drills (X₃): either single or twin drill. The dependent variables included % release at 2 h (Y₁), 4 h (Y₂), and 8 h (Y₃) noted Q2h, Q4h, and Q8h, respectively. In addition, the coefficient of determination, R², of fitting the data to zero-order release profile was chosen as an additional factor for comparison. All formulations contained 2.5 mg VNP/250 mg SNEDDS incorporated into the tablet core.

2.4. In vitro evaluation of VNP-SNEOPT

2.4.1. Thickness and diameter

The thickness and diameter of 10 tablets from each formula were measured using a Vernier caliper and the means ± standard deviation (SD) were calculated.

2.4.2. Weight variation after coating

After the core tablets were coated, the weight variation test was conducted by weighing 20 randomly selected tablets individually. The average weight (± SD) was calculated.

2.4.3. Friability

Ten tablets from each formula were accurately weighed and placed in the drum of a friability tester (Model FR1000, Copley Scientific Ltd., Nottingham, UK), which rotated at 25 rpm for 4 min. The tablets were then brushed and reweighed. The percentage loss in weight was calculated and taken as a measure of friability.

2.4.4. Hardness

Hardness was determined using six tablets from each formulation. Each time the tablet was placed between the two anvils of a digital tablet hardness tester (Version 4.22, Schleuniger Pharmatron Inc., Manchester, NH), force was applied to the anvils, and crushing strength that just caused the tablet to break was recorded. The average hardness expressed in Newton (N) (± SD) was calculated.
2.5. In vitro VNP release study

All release studies were carried out in USP apparatus II rotating paddle (Hanson Research Corporation, Chatsworth, CA) at 50 rpm and at 37 ± 0.5°C. The dissolution was carried out using 130 mL 0.1N HCl pH 1.2 (simulated gastric fluid) for the first 2 h. The medium was then changed to phosphate buffer pH 6.8 (simulated intestinal fluid) for the next 6 h by adding the required volume of 0.2 M tri-sodium orthophosphate (Jha et al. 2012). The exact total volume of release media (~200 mL) was recorded every time and was taken into consideration in the calculation of the amount of drug released at each time interval. Samples of 5 mL were withdrawn at predetermined time intervals (0.5, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 h) and analyzed spectrophotometrically at 269 nm using a UV–Visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). An equal volume of fresh medium maintained at the same temperature was then added. The test was run in triplicate.

2.6. Mechanism of VNP release

An ideal osmotic pump system should be able to release all of its drug content at a constant release rate (i.e. zero-order kinetics). In order to study the mechanism of drug release from VNP osmotic tablets, release data were analyzed according to zero-order, first-order, and Higuchi kinetic equations. The latter was applied to the first 60% drug released (Ribeiro et al. 2012). DDSolver add-in was used for modeling and comparison of drug release profiles (Zhang et al. 2010). The model with the highest coefficient of determination ($R^2$) was considered to be the best-fitting one.

2.7. Statistical analysis

The resulting data were analyzed by using SPSS software applying univariate analysis of variance. Post hoc multiple comparisons (Duncan test) were applied when necessary. Differences between formulations were considered to be significant at $p < 0.05$.

2.8. In vivo pharmacokinetic study in rabbits

2.8.1. Study design

A study was designed in order to compare the pharmacokinetic parameters of VNP from the optimized S-SNEOPT to the commercially available Vinporal® tablets (Amriya Pharmaceutical Industries) following single oral dosing administration and a wash out period of 1 week. Two-treatment, two-period, randomized cross-over design was adopted using six healthy male albino rabbits (2–2.25 kg) purchased from the laboratory animal center of the National Research Center (Cairo, Egypt). The experimental protocol was approved by the Animal Ethics Committee (Faculty of Pharmacy, Cairo University), and the handling of the animals was in accordance with international guidelines (European Commission 2010). The rabbits were divided into two groups of three rabbits; group I received the test formula and group II received the marketed product (Vinporal®). Rabbits were fasted overnight with water ad libitum. Tablets were swallowed intact by pushing them to the back of the pharynx with a flexible gastric tube. Then, ~10 mL of water was given orally with a syringe to avoid tablet adherence to the throat and to help swallowing.

2.8.2. Sample collection and analysis

Blood samples (3 mL) were taken at zero time (predose) and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10 and 24 h postdose. The samples were withdrawn via the marginal ear vein into heparinized tubes. Plasma was separated immediately by centrifugation at 3000 rpm for 10 min at 4°C (Centurion Scientific Ltd., West Sussex, UK) and stored frozen at −20°C pending VNP analysis. The liquid chromatography coupled with mass spectrometry (LC–MS/MS) method developed and validated by El-Dahmy et al. (2014) was used for the analysis of VNP in the collected samples.

2.8.3. Pharmacokinetic and statistical analysis

VNP pharmacokinetic parameters after oral administration of the developed formulae compared to the market product were determined by non-compartmental pharmacokinetic models using Thermo Scientific™ Kineta Software version 5 (Waltham, MA). The peak plasma concentration ($C_{\text{max}}$) and the time to reach $C_{\text{max}}$ ($t_{\text{max}}$) were obtained from the plasma concentration–time data. The area under the plasma concentration–time curve from time zero to the last time point and to infinity ($\text{AUC}_{0-\infty}$) were calculated using the linear trapezoidal rule. Mean residence time (MRT) was calculated from the terminal portion of the ln(concentration)–time curve using regression.

2.8.4. Establishment of in vitro/in vivo correlation

According to FDA guidelines, four categories of in vitro/in vivo correlation (IVIVC) can be established namely, levels A, B, C and multiple level C. The latter relates one or multiple pharmacokinetic parameters [such as $C_{\text{max}}, t_{\text{max}}, K_{\text{a}},$ or $\text{AUC}$ (total or cumulative)] to the amount of drug dissolved at different time points of the release profile (U.S. Department of Health and Human Services 1997). To estimate the multiple level C correlation, the cumulative AUCA–t was plotted against the percent of VNP released at the same time points and the coefficient of determination ($r^2$) was determined.

3. Results and discussion

3.1. Preliminary prepared VNP S-SNEOPT

The core tablets used in this study were prepared according to the composition developed in our previous work (El-Zahaby et al. 2016). The core tablets were then dip coated to produce the SNEOPT. Preliminary studies were performed to choose the proper polymers, polymer concentration and number of coat drills to be used in the coating process. The aim was to select the appropriate parameters to construct the factorial design to produce formulations with near zero-order release and high extent of drug release. Figure 1 illustrates the dissolution profile of some of the prepared formulae. All of the tablets did not release most of the drug included and the maximum amount released (58% after 8 h) was obtained from the tablets coated with 2.5% Opadry® CA and drilled twice. In general, VNP released from tablets was higher when the tablets were coated using 2.5% Opadry® CA by fixing the number of dippings into coating solution to 2 × 10s dippings compared to coating until a 10% weight gain was obtained. This is due to the lower amount of coat depositing on the surface of the tablets. Therefore, two times dippings into coating solution was the method of choice in further work. Also, since none of the prepared preliminary formulae reached full drug release, lower polymer concentration was adopted in the full factorial design to ensure complete drug release from the SNEOPT.

3.2. Quality control tests of VNP SNEOPT prepared using the factorial design

The diameter and thickness of the prepared VNP SNEOPT ranged between 12.02–12.05 mm and 0.811–0.814 mm, respectively. The weight gain per tablet reached after coating was found to be
between 0.01 and 0.02 g. The low concentrations used in coatings along with the short dipping time led to the small weight gain observed. The results of the friability and hardness tests are shown in Table 1. All 1.5% and 2% coated tablets passed friability test. On the other hand, all the 1% coated tablets did not pass friability test except the twin drilled 1% Opadry® CA coated tablets.

The low concentration coatings produced more fragile coats that peeled off easily from the surface of the tablets. Opadry® CA coated tablets had a uniform appearance and were less liable to friability. This correlated with hardness results where tablets coated with CA or the mixture of CA/PEG showed lower hardness values compared to the core tablet without coating. On the other hand, Opadry® CA coated tablets possessed hardness values comparable to the core tablet (the core C4 hardness was 38 N). Collectively, hardness of all coated tablets ranged from 21 to 42 N, which is acceptable (Mahalaxmi et al. 2009; Ketjinda et al. 2011).

### 3.3. Optimization of VNP release from osmotic tablets using full factorial design

In order to compare the prepared formulae, three response variables were selected; the percentage of drug released after 2, 4, and 8 h. These three points will help in describing the initial release as well as the rate and extent of drug release. The optimum target from osmotic pump tablets is to obtain a constant zero-order release independent of time and the conditions in the gastrointestinal tract (Shokri et al. 2008). However, one of the problems encountered with osmotic systems is the lag time associated with the initial stage of water imbibition inside the system to start the release. That is why it is beneficial to have a minimal initial drug release as denoted by $Q_{2h}$, when formulating extended release osmotic systems before majority of the drug being released in a specific time period (Huang et al. 2004).

#### 3.3.1. In vitro release profile of VNP from CA coated SNEOPTs

A reported drawback of osmotic pump tablets is the lag time encountered before drug release starts especially for water insoluble drugs. This problem also appears when dense tablet coating is used. As shown in Figure 2, the use of asymmetric membrane coating in the preparation of SNEOPT led to more rapid release in the initial phase and relatively higher $Q_{2h}$ values. This type of coating was known to have the ability to minimize the lag time encountered in the usual dense coatings allowing the drug to be released from numerous delivery ports (Herbig et al. 1995).

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### Table 1. Composition and responses of $3^2 \times 2^1$ factorial design.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Type of coating material ($X_1$)</th>
<th>Concentration of coating solution ($X_2$)</th>
<th>Number of drills ($X_3$)</th>
<th>Friability (%)</th>
<th>Hardness ($N\pm SD$)</th>
<th>$Q_{2h}$ ($Y_1$) (%±SD)$^a$</th>
<th>$Q_{4h}$ ($Y_2$) (%±SD)$^a$</th>
<th>$Q_{8h}$ ($Y_3$) (%±SD)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CA</td>
<td>1%</td>
<td>1</td>
<td>9.756</td>
<td>22.33 ± 0.58</td>
<td>28.65 ± 1.99</td>
<td>60.40 ± 1.00</td>
<td>94.27 ± 4.30</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>2%</td>
<td>1</td>
<td>12.195</td>
<td>21.00 ± 1.00</td>
<td>38.79 ± 4.45</td>
<td>61.20 ± 0.73</td>
<td>97.85 ± 0.11</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>1.5%</td>
<td>2</td>
<td>0.298</td>
<td>23.00 ± 1.00</td>
<td>34.18 ± 0.87</td>
<td>41.66 ± 1.60</td>
<td>60.93 ± 10.10</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>2%</td>
<td>0</td>
<td>24.67 ± 1.53</td>
<td>29.27 ± 0.72</td>
<td>41.55 ± 0.60</td>
<td>50.11 ± 2.16</td>
<td>62.01 ± 1.07</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>2%</td>
<td>0</td>
<td>24.33 ± 1.15</td>
<td>29.81 ± 3.88</td>
<td>40.12 ± 2.11</td>
<td>51.79 ± 1.07</td>
<td>63.01 ± 4.99</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>2%</td>
<td>1</td>
<td>0.123</td>
<td>22.00 ± 0.00</td>
<td>43.36 ± 6.08</td>
<td>75.50 ± 4.14</td>
<td>96.87 ± 0.01</td>
</tr>
<tr>
<td>7</td>
<td>CA/PEG (80:20)</td>
<td>1%</td>
<td>2</td>
<td>8.791</td>
<td>23.67 ± 1.53</td>
<td>36.43 ± 1.73</td>
<td>88.29 ± 2.61</td>
<td>96.54 ± 4.99</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>2%</td>
<td>2</td>
<td>8.363</td>
<td>23.67 ± 1.53</td>
<td>36.43 ± 1.73</td>
<td>88.29 ± 2.61</td>
<td>96.54 ± 4.99</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>1.5%</td>
<td>1</td>
<td>0.055</td>
<td>29.00 ± 0.00</td>
<td>43.36 ± 6.08</td>
<td>75.50 ± 4.14</td>
<td>96.87 ± 0.01</td>
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<tr>
<td>10</td>
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<td>2</td>
<td>0.552</td>
<td>29.33 ± 0.58</td>
<td>33.67 ± 0.96</td>
<td>48.11 ± 5.09</td>
<td>86.93 ± 4.29</td>
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<tr>
<td>11</td>
<td></td>
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<td>1</td>
<td>0.332</td>
<td>26.00 ± 2.00</td>
<td>22.40 ± 1.60</td>
<td>34.72 ± 3.00</td>
<td>47.12 ± 1.57</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>2%</td>
<td>2</td>
<td>0.111</td>
<td>24.33 ± 1.15</td>
<td>30.06 ± 4.86</td>
<td>42.66 ± 0.97</td>
<td>68.29 ± 4.82</td>
</tr>
<tr>
<td>13</td>
<td>Opadry® CA</td>
<td>1%</td>
<td>1</td>
<td>7.377</td>
<td>23.00 ± 0.00</td>
<td>42.05 ± 2.15</td>
<td>91.52 ± 0.46</td>
<td>100.58 ± 1.06</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>2%</td>
<td>0</td>
<td>0.114</td>
<td>24.00 ± 0.00</td>
<td>61.06 ± 0.49</td>
<td>84.57 ± 1.11</td>
<td>101.72 ± 3.34</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>1.5%</td>
<td>2</td>
<td>0.332</td>
<td>40.67 ± 0.58</td>
<td>41.80 ± 1.08</td>
<td>69.92 ± 0.66</td>
<td>101.62 ± 2.26</td>
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<tr>
<td>16</td>
<td></td>
<td>2%</td>
<td>1</td>
<td>0</td>
<td>38.00 ± 0.00</td>
<td>32.14 ± 0.15</td>
<td>47.12 ± 0.05</td>
<td>66.53 ± 0.06</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>2%</td>
<td>2</td>
<td>0.159</td>
<td>40.67 ± 1.53</td>
<td>35.49 ± 3.13</td>
<td>57.69 ± 8.25</td>
<td>98.79 ± 4.52</td>
</tr>
</tbody>
</table>

$^aQ_{2h}$, $Q_{4h}$, and $Q_{8h}$ are the % cumulative VNP released at 2, 4, and 8 h, respectively.
According to statistical analysis (Duncan test), there is no significant difference \( p > .05 \) in the overall % VNP released from the single and twin drilled formulae coated by either 1.5% or 2% CA. All those formulae released from 50% to 66% VNP after 8 h. Changing the number of drills in those membranes had no significant effect \( p > .05 \) on VNP release as well. These results are in accordance with the work of Derakhshandeh and Berenji (2014), where they tested the influence of the number of release orifices on the release of buspirone from oral osmotic pump tablets. Their results showed that the drug release rate did not increase significantly with an increasing number of release orifices from 1 to 2 \( p > .05 \) and it is concluded that the main force for drug release in the prepared osmotic tablets is the osmotic pressure across the coating membrane. The tablets coated with 2% CA remains intact throughout the release while the tablets coated with 1.5% CA showed a small rupture in the coat after 8 h. There was no significant difference \( p > .05 \) between the two 1% CA formulations; however, the extent of drug release was significantly higher \( p < .05 \) than the 1.5% and 2% CA SNEOPT. The tablet coating cracked shortly after the tablet was exposed to the dissolution medium. This was probably due to the lack of resistance of the thin layer of coating around the tablet, which was unable to tolerate the building hydrostatic pressure induced by polymer swelling (Shokri et al. 2008). This can also explain the non-significant difference between the single- and twin-drilled tablets. It is previously reported that when a low concentration of CA in the coating and/or a low coating level was applied to osmotic tablets, the produced asymmetric membrane tends to rupture during in vitro testing (Am Ende & Miller 2007).

3.3.2. In vitro release profile of VNP from SNE controlled porosity osmotic pump tablets coated with CA/PEG

The effect of changing the mixture of CA/PEG concentration and the number of drills on VNP release from the prepared controlled porosity osmotic pump tablets (CPOPTs) was investigated. PEG was used in many studies as a leachable pore-forming material to change the CA membrane permeability (Makhija & Vavia 2003; Abd-Elbary et al. 2011). Also, PEG acts as a plasticizer that enhances both the flexibility and the mechanical resistance of the membrane coating against internal osmotic pressure (Abd-Elbary et al. 2011; Adibkia et al. 2014).

As shown in Figure 3, 2% coated single drilled CPOPTs showed significantly lower \( p < .05 \) rate and extent (47% after 8 h) of VNP release among this category of coating; as the coating solution concentration increased, the drug release decreased. The higher polymer concentration led to lower membrane permeability to the dissolution medium. Therefore, VNP release was slower through the tablet coat. An inverse correlation between the rate of drug release and CA coated CPOPTs was observed in a study by Abd-Elbary et al. (2011). In addition, Shokri et al. (2008) reported that drug release rate decreased as a result of using thicker coating on CPOPTs which in turn decreased the rate of water penetration through the membrane.

There was no significant difference \( p > .05 \) between VNP release from 1% CA/PEG CPOPTs with 1 and 2 drills. The same was observed for 1.5% coated tablets. At lower CA/PEG concentrations (1% and 1.5%), the membrane permeability is already high due to the controlled pores formed in the coats. This led to decreased effect of the drills on membrane coat permeability. Also, a non-significant difference \( p > .05 \) between the mean VNP release from the CPOP tablets coated with 1.5% CA/PEG (single drill) and 2% CA/PEG (twin drills). The twin drilled membrane with higher polymer concentration (2%) gives the same results as when decreasing polymer concentration to 1.5% and make a single drill in the membrane. This indicates that when the coat permeability is already high due to presence of pore former, the resistance to water transport in the tablet core is more significant than the transport through the coat. This is in accordance with the work by Thombre et al. (2004), where changing the CA/PEG ratios between 7/3 and 6/4 yielded similar drug release profiles. Both osmotic tablets coated by either 1.5 or 2% CA/PEG remained intact during the 8 h period of the release test.

The fastest release rate was observed for 1% CA/PEG coated CPOPTs either having 1 or 2 drills, where they released the whole VNP content at 8 h. The membrane coating of these tablets was ruptured during the release study and the tablets disintegrated partially. This explains the complete VNP release within 8 h. Low polymer concentration along with the presence of the water soluble PEG made the membrane fragile and highly permeable. So, it was not able to withstand the pressure inside the osmotic tablets and ruptured in the dissolution medium releasing the VNP contents faster than other osmotic tablets under study in this group.

![Figure 2. In vitro release profiles for VNP core tablets coated with (2%, 1.5%, and 1%) asymmetric CA membrane by fixed number of dipping and either having 1 or 2 drills in 0.1 N HCl for 2 h followed by changing media to pH 6.8 phosphate buffer till 8 h at 37 ± 0.5 °C.](image-url)
From the previous results, it is clear that more VNP was released from CPOP than CA coated tablets. During dissolution, water is imbibed by the core from the dissolution medium across membrane. However, although both are asymmetric membranes, the pores formed in the membrane gradually following dissolution of PEG generated a more porous membrane that helps in releasing VNP out of the core at a higher rate (Verma et al. 2002).

### 3.3.3. In vitro release profile of VNP from SNEOPTs coated with Opadry® CA

As illustrated in Figure 4, 1.5% and 2% Opadry® CA coated tablets with a single drill showed the slowest VNP release; where they released 66.5% and 77.5% VNP, respectively, at 8 h. While 1% Opadry® CA coated SNEOPTs with twin drills released VNP rapidly compared to other formulations (101.4% at 8 h). As the concentration of Opadry® CA increased, VNP release was more controlled. The membrane of higher polymer concentration might represent a more resistant barrier to drug release than the one of lower polymer concentration. Increasing the polymer concentration in the coat will produce more resistance for the release medium to permeate inside the tablet and thus lower the dissolution rate of the tablet core components which consequently reduces drug release rate from osmotic tablets. These results are similar to those obtained by Abd-Elbary et al. (2011), where the CPOPT coated with solution of higher CA concentration showed more prolonged drug release rates than the formulae coated with solution of lower CA concentrations. There was a non-significant difference ($p < .05$) between VNP released from SNEOPTs coated with 1% (single drill) and 1.5% (twin drills) Opadry® CA. Decreasing the polymer concentration by 0.5%, which was expected to increase the rate of VNP release, was nullified by decreasing the number of drills. This highlights the importance of the number of drills for Opadry® CA as compared to the controlled porosity CA/PEG coat.

The coating of the 1% Opadry® CA SNEOPTs partially separated from the tablets’ core after 3 h, while a rupture was observed after 8 h for the twin drilled 1.5% Opadry® CA coated tablets. Twin drilled tablets coated with 2% Opadry® CA remained intact throughout the whole release study. The behavior of different coat concentrations in the release media during the test helped in explaining the differences and similarities in VNP released from
osmotic tablets. As long as the membrane kept its integrity (2% Opadry® CA), the VNP release was more controlled. The rupture or separation of membranes led to more VNP release. Therefore, the coating membrane composition is crucial to provide the convenient quantity of water in the tablet core in the appropriate time and to assure that the accumulated pressure does not lead to system rupture. Therefore, 1% Opadry® CA was excluded from further consideration.

To investigate the changes in the membrane structure, surface of the tablet’s coat (both before and after release studies) was studied using projection microscope.

Supplementary Figure S1(A) shows the photograph obtained from projection microscopy of the Opadry® CA asymmetric tablet’s coat before dissolution study revealing the pre-formed pores within the coat. In Supplementary Figure S1(B), after being in contact with the dissolution medium, the number of pores increased due to the presence of PEG in the composition of Opadry® CA, which created more pores upon contact with release medium. Many of these pores were either enlarged or inter connected to one another during the release study. Similar observations were reported by Okimoto et al. (1999) who concluded that the addition of micronized lactose as a pore former made the CA membrane surface more porous when examined by scanning electron microscopy. In addition, after dissolution, the exhausted membranes of the single drilled coated tablets were visually observed for any cracks in the coating. There were no visible cracks in the coating and they were intact after complete release. The integrity of the osmotic system is important to keep controlling VNP release through the SNEOPT.

### 3.3.4. Modeling of drug release profiles

Release data of the prepared formulations were fitted to various mathematical models (zero-order, first-order, and Higuchi diffusion models) in order to describe the kinetics of drug release. The coefficient of determination ($R^2$) was taken as a criterion for selecting the most appropriate model. The acceptable fit of the zero-order kinetic model to the drug release data indicates that a concentration independent mechanism is predominant (Barzegar-Jalali et al. 2008), which is the optimum target of preparing osmotic pump tablets.

All CA coated tablets followed Higuchi model (Table 2). This could be explained based on the asymmetric membrane nature used in the study. The produced porous membrane upon contact with the dissolution media makes the drug diffusion through the created pores the predominant pathway. Similarly, Bi et al. (2007) concluded that the drug release from the porous osmotic systems of theophylline is influenced by micro-environmental osmotic pressure. This pressure is produced by the osmotic agents’ dissolution in the water permeating through the membrane and the diffusion through the pores produced by the pore formers. According to Table 2, 6 out of the 18 formulae showed zero-order release kinetics. This suggests that the rate of water imbibition across the coating membrane was perfectly controlled so that a saturated solution of sodium chloride in the tablet core was maintained. This would lead to a constant osmotic pressure gradient between the tablet core and the external environment throughout the release tests durations (Abd-Elbary et al. 2011). The highest $R^2$ value (0.998) was corresponding to the twin drilled tablets coated by 2% Opadry® CA but those tablets released the whole VNP contents (98.793%) in 8 h. On the other hand, tablets coated by 1.5% Opadry® CA and having a single drill had $R^2$ equal to 0.993 and VNP release was continued until 24 h (data not shown) which matched the aim of formulating once daily controlled release formulation. Based on these results, single drilled tablets coated by 1.5% Opadry® CA was selected as the optimum VNP SNEOPT formulation.

### 3.3.5. Comparison of the in vitro release profile of the selected optimum formula and the reference market product (Vinporal® tablet)

VNP release from the marketed tablet was complete within the first 30 min of dissolution in acidic medium (pH 1.2) because of its weakly basic nature. Therefore, it was not possible to proceed on the following steps of the dissolution experiment where the pH is changed. This result is in accordance with that found by Ribeiro et al. (2007) where VNP release from the instant release formula was immediate in the first hour of the dissolution at pH 1.2. Based on this result, the in vitro release of the marketed product was made separately in 0.1N HCl (pH 1.2) and then in phosphate buffer (pH 6.8).

Figure 5 shows a comparison between the in vitro release profiles for VNP single drilled tablets coated with 1.5% Opadry® CA which is the selected optimized formulation, and that of the marketed product in both 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8). It is obvious that, the marketed product showed rapid

### Table 2. Collective squared correlation coefficient ($R^2$) of zero-order, first-order and Higuchi models.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Type of coating material ($X_1$)</th>
<th>Concentration of coating solution ($X_2$)</th>
<th>Number of drills ($X_3$)</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CA</td>
<td>1%</td>
<td>1</td>
<td>0.917</td>
<td>0.894</td>
<td>0.942</td>
</tr>
<tr>
<td>2</td>
<td>CA</td>
<td>2%</td>
<td>2</td>
<td>0.955</td>
<td>0.889</td>
<td>0.972</td>
</tr>
<tr>
<td>3</td>
<td>1.50%</td>
<td>1%</td>
<td>1</td>
<td>0.905</td>
<td>0.933</td>
<td>0.962</td>
</tr>
<tr>
<td>4</td>
<td>2%</td>
<td>1%</td>
<td>1</td>
<td>0.952</td>
<td>0.944</td>
<td>0.962</td>
</tr>
<tr>
<td>5</td>
<td>1%</td>
<td>2%</td>
<td>1</td>
<td>0.882</td>
<td>0.924</td>
<td>0.957</td>
</tr>
<tr>
<td>6</td>
<td>CA/PEG (80:20)</td>
<td>1%</td>
<td>1</td>
<td>0.915</td>
<td>0.957</td>
<td>0.981</td>
</tr>
<tr>
<td>7</td>
<td>CA</td>
<td>1%</td>
<td>2</td>
<td>0.932</td>
<td>0.971</td>
<td>0.972</td>
</tr>
<tr>
<td>8</td>
<td>CA</td>
<td>1%</td>
<td>2</td>
<td>0.868</td>
<td>0.873</td>
<td>0.914</td>
</tr>
<tr>
<td>9</td>
<td>1.50%</td>
<td>1%</td>
<td>1</td>
<td>0.981</td>
<td>0.914</td>
<td>0.942</td>
</tr>
<tr>
<td>10</td>
<td>2%</td>
<td>2%</td>
<td>1</td>
<td>0.976</td>
<td>0.849</td>
<td>0.952</td>
</tr>
<tr>
<td>11</td>
<td>1.50%</td>
<td>2%</td>
<td>1</td>
<td>0.910</td>
<td>0.947</td>
<td>0.968</td>
</tr>
<tr>
<td>12</td>
<td>Opadry® CA</td>
<td>1%</td>
<td>2</td>
<td>0.963</td>
<td>0.991</td>
<td>0.988</td>
</tr>
<tr>
<td>13</td>
<td>CA</td>
<td>1%</td>
<td>2</td>
<td>0.980</td>
<td>0.790</td>
<td>0.933</td>
</tr>
<tr>
<td>14</td>
<td>CA</td>
<td>2%</td>
<td>1</td>
<td>0.966</td>
<td>0.945</td>
<td>0.985</td>
</tr>
<tr>
<td>15</td>
<td>Opadry® CA</td>
<td>1%</td>
<td>2</td>
<td>0.993</td>
<td>0.970</td>
<td>0.975</td>
</tr>
<tr>
<td>16</td>
<td>2%</td>
<td>1%</td>
<td>1</td>
<td>0.989</td>
<td>0.967</td>
<td>0.960</td>
</tr>
<tr>
<td>17</td>
<td>1.50%</td>
<td>2%</td>
<td>1</td>
<td>0.957</td>
<td>0.994</td>
<td>0.993</td>
</tr>
<tr>
<td>18</td>
<td>Opadry® CA</td>
<td>2%</td>
<td>2</td>
<td>0.998</td>
<td>0.982</td>
<td>0.959</td>
</tr>
</tbody>
</table>

*The model with the highest $R^2$ value is shown in bold case for each formulation.*
release in acidic medium (102.258% in 30 min). Based on data in the literature, a sharp decrease in VNP solubility and dissolution at higher pH values was expected. This was clear in the dissolution curve of marketed VNP in phosphate buffer pH 6.8 media where, only 11.741% of drug was released after 8h. Hence, S-SNEOPT was developed to improve VNP dissolution behavior over a pH range that simulates the fasted GIT. The dissolution profile of the single drilled VNP tablets coated by 1.5% Opadry® CA clearly indicated a controlled release pattern over 8 h of the experiment, where 77.576% VNP was released. Along with optimized formulation parameters; concentration and type of coat and number of drills that yield zero-order kinetics, the presence of HPMC K4M led to tablet swelling upon contact with the dissolution media and a gel layer was formed on their surfaces. This gel delayed water penetration into the tablet and thus reduced drug release rate. Still, the extent of drug release was nearly complete at the end of the dissolution experiment (after 24h, data not shown). The incorporation of VNP into the S-SNEDDS helped in achieving a constant drug release in both media (pH 1.2 and 6.8) because of the pH independent solubility and dissolution rate. Also, the disadvantage faced with osmotic pump tablets when lipophilic drugs are formulated and are difficult to be released completely was resolved.

3.4. In vivo pharmacokinetics study in rabbits

The pharmacokinetic parameters of VNP following oral administration of the immediate release Vinporal® tablet as well as the controlled release optimized VNP SNEOPT were evaluated in rabbits. Figure 6 illustrates the mean plasma concentration–time profile of VNP following the administration of the optimized SNEOPT and the marketed product.

Table 3 shows a summary of the pharmacokinetic parameters obtained by non-compartmental fitting of the concentration time data of VNP following the oral administration of Vinporal® and the optimized VNP SNEOPT. Statistical analysis of the obtained data revealed non-significant differences ($p > .05$) between $C_{\text{max}}$ and $AUC_{0-\infty}$ for the tested formulae. Whereas the $AUC_{0-\infty}$ of VNP following the administration of the osmotic tablets showed significantly higher ($p < .05$) extent of VNP absorption when compared to the marketed product Vinporal®.

Significantly lower median $t_{\text{max}}$ ($p < .05$) observed for the osmotic tablet was due to the high initial drug release achieved and the enhancement of VNP solubility by incorporation into S-SNEDDS. The spontaneous formation of VNP loaded nanoemulsion in the GI tract presented the drug in a solubilized form inside the nanosized oil droplets, which possess large interfacial surface area for drug absorption (Kang et al. 2004).

The significant prolongation ($p < .05$) of median MRT following the administration of VNP osmotic tablets showed evidence for the controlled VNP release achieved by the optimized SNEOPT when compared to the marketed product. This results was also observed by Abd-Elbary et al. (2011), where a prolonged MRT from 6.62 to 12.12 h was achieved by the etodolac CPOP tablets compared to Napilac® capsules.

The relative bioavailability was found to be 236.048% for the tested VNP osmotic tablet, based on the mean $AUC_{0-\infty}$ of the

![Figure 5. Comparison between the in vitro release profiles of VNP from single drilled osmotic tablets coated with 1.5% asymmetric Opadry® CA membrane and the marketed tablet Vinporal® (in 0.1 N HCl and pH 6.8 phosphate buffer).](image)

![Figure 6. Mean plasma concentration–time curve following the oral administration of the reference Vinporal® tablets and the osmotic tablets of the optimized formulation of VNP.](image)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Vinporal® tablet</th>
<th>VNP osmotic tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>17.831 ± 8.381</td>
<td>23.862 ± 3.011</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)$^b$</td>
<td>2.50</td>
<td>0.25</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (ng h/mL)$^b$</td>
<td>112.525 ± 17.579</td>
<td>378.42 ± 100.033</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (ng h/mL)$^b$</td>
<td>160.314 ± 17.016</td>
<td>378.42 ± 100.033</td>
</tr>
<tr>
<td>MRT (h)$^b$</td>
<td>21.28</td>
<td>50.41</td>
</tr>
</tbody>
</table>

$^a$Median.  
$^b$Significant difference ($p < .05$).
tested formula compared to that of the reference standard marketed product which represents approximately twofold improvement. The possible reasons of the lower bioavailability of VNP from the marketed product were its poor water-solubility, and extensive metabolism in the liver (Chen et al. 2008). The substantial bioavailability enhancement of VNP from S-SNEDDS in the current study can be attributed to its promotion of lymphatic transport through the transcellular pathway due to the presence of the oily phase Maisine™ 35–1 (Haus et al. 1994). Long-chain oils like Maisine™ 35–1 promote the lipoprotein synthesis, an effect similar to the presence of food, that could apparently enhance the bioavailability of VNP (Charman & Stella 1991; Chen et al. 2008). Additionally, VNP is a highly lipophilic compound so it has good solubility in triglycerides oils. Consequently, the aforementioned factors might contribute toward absorption via the lymphatic route, minimizing VNP first-pass effect.

Moreover, the high content of Cremophor® EL with intermediate HLB value in the composition of S-SNEDDS improves VNP bioavailability by enhancing drug dissolution (Chen et al. 2008) and increasing intestinal epithelial permeability by disturbing the cell membrane that facilitated the transcellular absorption (Swenson & Curatolo 1992). In addition, surfactant monomers can form polar defects in the lipid bilayer by partitioning into the cell membrane. The membrane could be dissolved into surfactant–membrane mixed micelles at high surfactant concentrations in the cell membrane (Swenson & Curatolo 1992; Kommuru et al. 2001). It was also reported that Cremophor® EL can reversibly open the tight junctions in the intestinal wall leading to paracellular transport (Lindmark et al. 1995). Additionally, it was reported that it has an effect in decreasing p-glycoprotein drug efflux (Nerurkar et al. 1996). The S-SNEDDS contains also the absorption enhancer, Transcutol® HP, which may as well have contributed to the achieved enhancement in VNP bioavailability (Cui et al. 2005).

It was also reported that the SNEDDS can modify hydrogen bonding and ionic forces between the polar head groups of the lipid bilayers. Also, SNEDDS can disrupt the membrane lipid-packaging arrangement by inserting itself between the lipophilic tails of the bilayers. Previous caco-2 cells studies indicated that VNP-SNEDDS was able to open the intestinal tight junctions as opposed to VNP powder (Chen et al. 2008).

3.5. Establishment of IVIVC

IVIVC is an important method to determine the ability of the in vitro dissolution method to predict the in vivo performance of the tested drug formulation. If established, the in vitro method can be used as a replacement for bioequivalence studies (El-Mahrouk et al. 2014). In Figure 7, the multiple level C linear plot of the cumulative AUC_{0-7} versus the percent of VNP dissolved at the same time points is illustrated. Good point to point relationship was established. A coefficient of determination of 0.9748 was obtained which indicate a close linear correlation.

4. Conclusions

Osmotic VNP formulation containing the drug in the form of S-SNEDDS was successfully designed to overcome VNP solubility drawback at higher pH values, extend drug release for a longer period of time without the risk of precipitation, and enhance drug bioavailability. In vitro release of the optimized formula was successful in reaching complete VNP release at a zero-order rate throughout the release study. The use of asymmetric membrane coating was successful in reaching more complete VNP release when compared to dense coating. The presence of pore forming PEG in the coating material had a prevalent effect in enhancing VNP release when compared to the effect of the number of drills. In vivo experiment in rabbits showed approximately twofold increase in bioavailability of VNP from the optimum SNEOPT compared to the marketed conventional VNP tablet.

Disclosure statement

No potential conflict of interest was reported by the authors.

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