Delivery of a CNS Acting Drug *via* the Development of an Osmotic System

A Thesis Presented By

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For The Degree of Doctor of Philosophy in Pharmaceutical Sciences

"PHARMACEUTICS"

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2015

Abstract

Oral route is the most extensively used route of drug administration because of its ease of administration that improved patient compliance. The osmotic-controlled release oral delivery system, OROS, is an advanced drug delivery technology that uses osmotic pressure as the driving force to deliver the drug in a sustained manner independent of the drug chemical properties, the patient's physiological factors or concomitant food intake.

Osmotic drug delivery systems consist of a tablet core that is coated with a semi-permeable membrane coating. This coating has one or more delivery ports through which a solution or suspension of the drug is released over time.

Self-emulsifying drug delivery systems (SEDDS) are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents. Upon mild agitation followed by dilution in aqueous media, such as gastrointestinal fluids, these systems can form fine oil in water (O/W) emulsions. Solid SEDDS (S-SEDDS) is one of the lipid-based drug delivery systems prepared by the incorporation of liquid excipients into powders by solidification. It is a promising drug delivery system for poorly water soluble compounds as it combines the advantages of liquid SEDDS (solubility and bioavailability enhancement) with those of solid dosage forms (high stability with various dosage forms options).

Vinpocetine (VNP) is a semi-synthetic derivative of vincamine; an alkaloid derived from the plant Vinca minor L. belonging to the periwinkle family. VNP is insoluble in water, (the water solubility ≈5 μg/ml), with a short half-life time of about 1-2 h and it is cleared by extensive metabolism in liver. It is mainly absorbed from the small intestine and its oral bioavailability in human is as low as 7% under fasting condition which limits greatly its clinical use. Therefore, preparing self-nano-emulsifying drug delivery system (SNEDDS) of VNP will lead to a spontaneous

formation of nano-emulsion upon drug release from the dosage form which will present the drug in a dissolved state and the small droplet size will provide a large interfacial surface area favored for drug absorption. Drug will also be accumulated in Peyer's patches for its lymphatic transport. In addition, due to its minimal half-life, VNP is a good drug for sustained release dosage form.

The work in this thesis is divided into:

Chapter 1: Solid self-nano-emulsifying drug delivery system of Vinpocetine (VNP-S-SNEDDS)

Chapter 2: Formulation and optimization of Vinpocetine solid-self-nano-emulsified osmotic pump–based controlled release systems

Chapter 3: *In vivo* performance of the optimized Vinpocetine solid-self-nano-emulsified (VNP S-SNE) osmotic system

<u>Chapter 1:</u> Solid self-nano-emulsifying drug delivery system of Vinpocetine (VNP-S-SNEDDS)

The aim of this chapter is to formulate and evaluate the S-SNEDDS of VNP and then transforming it into tablet that act thereafter as the core for the osmotic system. Two liquid systems were tried and the successful one which consisted of 10% Maisine™ 35-1 (oil), 50% Cremophor® EL (surfactant), and 40% Transcutol® HP (co-surfactant, solubilizer and absorption enhancer) loaded with VNP was used. Every 250 mg of this system contained 2.5 mg VNP. Evaluation of the liquid VNP SNEDDS was performed by many tests. Assessment of the self-emulsification efficiency of the prepared SNEDDS was tested visually by the aid of a standard USP dissolution tester. The emulsification time, which is the time needed for SNEDDS to form homogeneous transparent mixture upon dilution, was also monitored. The emulsification time in 0.1 N HCl was 12 sec. Time required for the complete disappearance of SNEDDS in distilled water under the same condition was

14 sec. The VNP-loaded system was equilibrated at both ambient temperature and 4 °C for 1 week. The system showed neither phase separation nor drug precipitation under both conditions. The droplet size and polydispersity index (PDI) of the formulation were determined by Zeta sizer Nano ZS. It was found that the freshly prepared liquid SNEDDS exhibited small particle size from 16.62 to 17.35 nm and the value of PDI of the sample was found to be in the range 0.045-0.128, suggesting that upon dilution with body fluids, the nano-emulsion remains stable and will not convert into macro-emulsion. Average zeta potential was -12.65 ± 4.39 mV. The nanostructure and surface morphology of nano-emulsion produced from the reconstituted liquid SNEDDS were examined by transmission electron microscopy (TEM). Spherical droplets of average size of 17.62 ± 2.12 nm were obtained. The reconstituted and liquid system were examined physically against distilled water for any turbidity and it was observed that both preserved their clear appearnace during the 6 months of the stability test.

Preparation of S-SNEDDS of VNP was done by using freeze-drying or lyophilization but results were not successful. Therefore, another method was adopted which was adsorption onto solid carriers by mixing in mortar, the later, produced free flowing powders after changing the carrier and excipients until reaching the best combination. Aeroperl® was the carrier of choice that produced best VNP-S-SNEDDS. Solid state characterization of VNP-S-SNEDDS was also performed by many tests like; measuring the nano-emulsion globule size, PDI and Zeta potential of the reconstituted S-SNEDDS. The particle size analysis of the reconstituted S-SNEDDS reveals comparative results to the liquid SNEDDS, where size of globules was in range of 16.57-17.53 nm. PDI was found in range of 0.385-0.823 and zeta potential was in range of -12 to -14.3 mV. Nano-emulsions produced from

the reconstituted S-SNEDDS were examined by TEM and showed spherical droplets of an average size of 15.55 ± 2.31nm. Therefore, transforming the liquid SNEDDS into S-SNEDDS by adsorption onto Aeroperl® did not seem to have a remarkable effect on droplet size nor the shape of the obtained droplets. The S-SNEDDS preserved the self-emulsification performance of the liquid SNEDDS.

Many trials have been made to formulate the core tablet based on the chosen system (250 mg liquid system loaded with 2.5 mg VNP). Powder mixtures with excellent flow properties were formulated, which upon compression into tablets produced ones with acceptable post-compression parameters. The cores C1-C4 (composed of the S-SNEDDS containing VNP with different tablets excipients) had diameter of 12 mm and they all passed the friability test (friability test results were in range from 0-0.5%). C1 and C4 showed almost the same pre and post-compression characteristics. During tableting procedure, C4 which contained Lubripharm® as lubricant was better in handling and its powder mixture was non-sticky. Based on these criteria, C4 was selected as best core to be used further to produce osmotic tablets of VNP.

Drug-excipients compatibility was assured by FT-IR and DSC studies. The spectrum of pure VNP showed its characteristic peaks at 1716 cm^{-1} which is the characteristic shoulder of carbonyl stretching bond (C=O) present in VNP and at 3000-2800 that is assigned to the aromatic stretching. The IR spectrum of the core containing VNP showed the characteristic peaks of the drug which indicated that there was no interaction between the drug and the used excipients. The DSC thermogram of pure VNP powder showed a melting endotherm at 154.8 °C. This matched the reported thermogram of the drug in literature. The DSC scan of the S-SNEDDS showed the absence

of a drug peak suggesting that VNP was completely dissolved in the oil system and adsorbed within the carrier inside the S-SNEDDS matrix.

<u>Chapter 2:</u> Formulation and optimization of Vinpocetine solid-self-nano-emulsified osmotic pump–based controlled release systems

The aim of this chapter is to formulate an osmotic system of VNP. A $3^2 * 2^1$ full factorial design was selected for optimization of VNP formulation. It consisted of eighteen full factorial design points. The independent variables selected for this study were: type of coat (X_1) concentration of coat (X_2) and number of drills (X_3). The dependent variables included % release at 2h (Y_1), at 4h (Y_2) and at 8h (Y_3).

All dissolution studies were carried out in USP paddle apparatus with rotating speed and temperature set at 50 rpm and 37±0.5°C, respectively. The used dissolution media were 0.1 N HCl (pH 1.2, 130 ml) for the first 2 h, then media were changed into phosphate buffer (pH 6.8) for the next 6 h. 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 distinct time intervals and analyzed for the drug concentration spectrophotometrically. The concentrations of drug samples and then the cummulative percentages of drug released were calculated according to equations derived from calibration curves constructed for each pH medium. An ideal osmotic system should be able to release a high percentage of drug content with a constant release rate (zero order kinetics) during 24 h. In order to study the accurate mechanism of drug release from VNP's osmotic tablets, drug release data were analyzed according to zero order, first order, and Higuchi kinetic equations. The model with the highest coefficient of determination (R^2) was considered to be the best fitting one.

The core tablet (C4) selected in Chapter 1 was coated by the dip-coating technique. Different coatings were tried until reaching the best coat's type and concentration, in addition to, best number of drills that yielded zero order release kinetics.

The elementary osmotic pump (EOP) tablets coated with 10% CA showed the most controlled drug release among the tested tablets; it released around 24% of VNP after 8 h. This tablet remained intact after being in contact with the release media for 24 h. Opadry® CA consisted of a mixture of CA with PEG 3350 in 80:20 ratio, respectively, showed faster VNP release than that obtained from tablets coated with CA but the drug release was still very slow.

In an attempt to increase coating permeability, asymmetric membrane was used. The tablets were dip-coated in polymer solutions consisted of CA, Opadry® CA or mixture of CA and PEG dissolved in a solution of acetone and a non-solvent, glycerol. The polymer-coating solution consisted of 1, 1.5 or 2 %W/V polymer and 10 %W/V glycerol, dissolved in acetone. After the tablets were coated with the polymer-coating solution, they were air-dried for 5 sec and then immersed in a water quench bath for 3 min. After removal from the water quench bath, the tablets were then air-dried under ambient conditions for at least 12 h.

During the *in vitro* release test, all tested formula showed a burst release which was reported to be one of the advantages of the asymmetric membrane coating. This type of coating was known to minimize the time lag before drug delivery begins and allow the drug to be released from a large number of delivery ports. Unfortunately, the use of 10% increase in tablet weight during coating procedures by different polymers concentrations had led to nearly similar release profiles. Many membrane concentrations were tried based on fixing the number of dipping during coating process to be

only 2 times dipping of tablet. The time of every dip was fixed to be 10 sec. Based on the *in vitro* release results of the preliminary coated tablets, a $3^2 * 2^1$ full factorial design (eighteen runs) was constructed. Herein, the effect of three levels of variables from a lower concentration to a higher concentration of 3 types of coats (1, 1.5 and 2%); CA, CA+PEG and Opadry® CA were selected and studied. The tablets were having either 1 or 2 drills.

According to statistical analysis (Duncan test), there was no significant difference in the overall % VNP released from the single and twin drilled formulae coated by either 2% or 1.5% CA. All those formulae released from 50-66% after 8 h. There was no significant difference between the two formulations (1% CA having 1 & 2 drills). The two formulations released almost the whole VNP content at 8 h.

The tablets coated by 2% (CA+PEG) and having a single drill controlled VNP release the most among this group of formulations. There was no significant difference between the two VNP tablets coated by 1.5% either having 1 or 2 drills according to Duncan test. In addition, there was no significant difference between the tablets coated by 2% (CA+PEG) and had 2 drills and those coated by 1.5% (CA+PEG) and had a single drill. The least controlled VNP release was observed in case of tablets coated by 1% (CA+PEG) either having 1 or 2 drills, where they released the whole VNP content at 8 h.

The tablets coated by either 2 or 1.5% Opadry® CA and having a single drill showed the most controlled VNP release. While those coated by 1% Opadry® CA and having twin drills released VNP rapidly compared to other formulations. The osmotic tablets coated by 1.5% Opadry® CA and having 2 drills is insignificantly different with those coated by 1% of the same coat and having only 1 drill according to Duncan test.

As a general conclusion, as the concentration of coat increased the drug release was decreased. Increasing the polymer concentration had led to a more resistant membrane for water permeability, and thus had lowered the dissolving rate of the tablet core components which consequently reduced drug release rate from osmotic tablets. Therefore, the SPM composition is crucial to provide the convenient quantity of water in the tablet core in the appropriate time and to assure that the pressure produced during swelling does not lead to rupture of the system so its optimization is very important for approaching a desirable release profile.

Six out of the 18 formulae showed zero order release kinetics. The highest R^2 value (0.9979) was belonging to the twin drilled tablets coated by 2% Opadry® CA but those tablets released the whole VNP content (98.79%) in 8 h. On the other hand, tablets coated by 1.5% Opadry® CA and having a single drill had R^2 equal to 0.9931and VNP release was continued until 24 h (97.079%) 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 best VNP SNE-OROS formulation that will be further *in vivo* tested.

A comparison between the *in vitro* release profiles of the selected VNP single drilled tablets coated by 1.5% Opadry® CA, and that of the marketed product in both buffer (pH 6.8) and 0.1 N HCl (pH 1.2) was performed. The results showed that the marketed product had very fast release in acidic medium (pH 1.2) that the total drug content was released in 30 min. On the other hand, the release profile of marketed VNP in buffer media (pH 6.8) showed that only 11.74% of drug is released after 8 h. Therefore, S-SNE osmotic tablet was attempted to improve the release performance of the drug over a pH range that simulates the one found in the fasted GIT. The release 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 77.57% VNP were released. Hence, osmotic VNP formulation containing the drug in the form of S-SNEDDS was designed to overcome the VNP solubility drawback at higher pH values and to extend drug release for a longer period of time without the risk of precipitation.

Chapter 3: ***In vivo*** **performance of the optimized Vinpocetine solid-self-nano-emulsified (VNP S-SNE) osmotic system**

The aim of this study involves the *in vivo* assessment of the aforementioned *in vitro* improvement in VNP's aqueous solubility along with checking the enhancement of the oral bioavailability of VNP through its incorporation into SNEOPT.

Six healthy male albino rabbits were used to perform the test. *In vivo* study was designed as a two-way randomized crossover study with a single oral dosing administration and a wash out period of one week. Blood samples were taken at zero time (pre-dose) and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10 and 24 h post dose. The samples were heparinized and the plasma of each was separated. The drug concentration in plasma was determined using a sensitive, selective and accurate LC–MS/MS method. Pharmacokinetic analysis included the calculation for each rabbit of the area under the curve to the last quantifiable concentration; AUC_{0-t}, and to infinity $AUC_{0-\infty}$, C_{max}, t_{max}, MRT, and $t_{1/2}$.

The results of this chapter showed the following:

The method was selective for VNP with minimum limit of quantification of 0.5 ng/ml. The LC–MS/MS assay has a good linearity from 0.5 ng/ml to 500 ng/ml. The standard plot obtained for plasma samples was linear in this concentration range and the linear regression analysis of the standard calibration plot for rabbit plasma was $y = 0.007104\ x + 0.00268$;

where y and x are the peak area ratio and VNP concentration, respectively. The small intercept indicates that the blank plasma has negligible interferences for the drug by its metabolites. The correlation coefficient of the line was 0.996. After calculation of the plasma concentration time data of rabbit no. 6, it was found that its results were out of range for all data points and for both the tested formulation and marketed product. So the data of rabbit no. 6 were omitted from further analysis and the given results are those for the remaining five rabbits. Higher C_{max} and lower t_{max} were observed for the VNP optimized osmotic tablet which might be due to the burst drug release achieved by using the asymmetric membrane coating that was clearly observed during the *in vitro* release test. The SNEDDS effect in enhancement of VNP solubility might represents another reason for these results. The mean $AUC_{0-\infty}$ of VNP following the oral administration of Vinporal® tablets and the VNP osmotic tablets were found to be 160.314 ± 17.016 ng.h/ml and 378.42 ± 100.033 ng.h/ml, respectively. This difference is proved to be statistically significant at $P<0.05$. This higher $AUC_{0-\infty}$ means a higher extent of drug absorption was achieved by the optimized formulation. The prolongation of both the mean elimination half-life ($t_{1/2}$) and mean residence time (MRT) of the drug led to a conclusion that the optimized osmotic tablet successfully controlled VNP release compared to the marketed Vinporal®. The relative bioavailability of the optimized VNP S-SNE osmotic tablet to Vinporal® tablet was 236.048%. The improvement in VNP bioavailability may be attributed to the following combined factors; formation of nano-emulsion in the GIT, presence of high content of Cremophor® EL, use of long chain fatty acid that promoted lymphatic absorption, and the presence of the absorption enhancer Transcutol® HP. Therefore, the successful VNP S-SNE osmotic tablet is expected to be an addition to formulations intended to improve the clinical outcomes of VNP by improving its oral bioavailability.