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Original Article

# Development and Evaluation of Self Micro-Emulsifying Formulation of Venlafaxine HCl with Improved Antidepressant Activity

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## ABSTRACT

**Aim/Background:** Venlafaxine HCl (VEN) is an antidepressant drug with extensive first pass metabolism and low oral bioavailability. In the present study, a solid self-emulsifying formulation of Venlafaxine was developed and evaluated for *in vitro* and *in vivo* performance. **Materials and Methods:** Various oils, surfactants, and cosurfactants were screened for the solubility of Venlafaxine in them. Surfactants were further screened based on their ability to emulsify oils. For the SMEDDS formulation, Caprol PGE 860 was selected as oil base, Tween 20 as a surfactant and propylene glycol as a co-surfactant. Pseudoternary phase diagrams were plotted with different concentrations of these three components with water to identify microemulsion area. The optimized formulation containing Venlafaxine (5.9%), Caprol PEG 860 (9.3%), Tween 20 (42.4%) and propylene glycol (42.4%) was converted into solid SMEDDS (S-SMEDDS) by using Spray Drying method and evaluated for the flow property, drug content, drug release, DSC, XRD, SEM and *in vivo* antidepressant activity. **Results:** The optimized SMEDDS formulation demonstrated globule size of 21 nm with no signs of precipitation upon dilution, was thermodynamically stable, and released more than 90% of Venlafaxine in 30 min. S-SMEDDS formulation was free flowing and SEM and PXRD studies revealed amorphous state of S-SMEDDS. *In vivo* antidepressant activity by forced swim test and anti-anxiety test by EPM maze test in rats demonstrated significant efficacy of SMEDDS formulation over plain drug. **Conclusion:** A self-emulsifying formulation of Venlafaxine was successfully developed and showed improved antidepressant activity in comparison to pure drug, thus can serve as potential alternative to existing oral formulations.

**Keywords:** Venlafaxine, Self-microemulsifying drug delivery system, Anti-depressant, First pass effect, Bioavailability, Solubility.

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## INRODUCTION

Depression is an extremely prevalent and recurrent condition affecting all aspects of human life. Monoamine hypothesis suggests that depression can be a result of a deficiency in neurotransmission of serotonin or norepinephrine in the brain.[1,2] Five major categories of antidepressants are effectively used for the treatment of depression, viz. Non-selective monoamine reuptake inhibitors, Selective noradrenaline reuptake inhibitors, Selective serotonin reuptake inhibitors, Monoamine oxidase inhibitors and atypical anti-depressants.[3,4]

Venlafaxine HCl (VEN) is a selective noradrenaline reuptake inhibitors class of antidepressant used to treat anxiety disorders and clinical depressions. Despite not recommended as a first line treatment, VEN is often effective for depression not responding to SSRIs. VEN is responsible for inhibiting the reuptake of serotonin and norepinephrine, thus potentiates neurotransmitter activity in the central nervous system.[5,6] VEN is a class I drug with high solubility and permeability and has logP of 2.69. Since the drug undergoes hepatic first pass metabolism, its oral bioavailability is only 45%.[7] VEN is a substrate of P-glycoprotein and studies reports its lower brain uptake due to efflux by p-glycoprotein transporters.[8] Attempts have been made to formulate intranasal nanostructured lipid carrier[9] and solid lipid nanoparticle formulation[8] of VEN to improve brain uptake. In market, immediate release and extended release oral formulations of VEN are available. However, no attempts have been made to address the low bioavailability of VEN.

Lipid-based delivery systems have been extensively explored for addressing the low bioavailability of drugs.[10,11] Several studies have revealed that lipid containing formulations have the ability

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to improve drug bioavailability and therapeutic efficacy, by the following proposed mechanisms.[12,13]

(a) Formation of colloids such as vesicles, mixed micelles, and micelles by stimulating biliary secretion, which further results in micellar solubilization of the drug in the intestinal fluid;

(b) Inhibition of drug efflux from enterocytes and of metabolism in enterocytes by Cytochrome P450-4A by interfering with enterocyte-based transport and metabolic processes.

(c) Selective lymphatic uptake, by which intestinal lymph is transported directly into the systemic circulation, decreasing first-pass drug metabolism.

In recent years, with the development of nanotechnology, most of the research is focused on the development of lipid based nanocarrier systems. Self-Microemulsifying Drug Delivery System (SMEDDS) is the most popular lipid nanocarriers with more stability, scalability and easier to formulate compared to other nanocarrier systems. SMEDDS are simply made by mixing various oils with cosolvents and lipophilic or hydrophilic surfactants, which get emulsified when exposed to fluids from the GIT, resulting in oil-in-water emulsions or microemulsions. Due to smaller size of resulting microemulsion (10 nm to 100 nm), drug is presented in form of molecular dispersion in GIT. As a result of its greater surface area, resultant microemulsion provides a large interfacial surface to promote lipolysis (lipolysis is an interfacial process), thereby increasing the rate of the process, increasing drug absorption and bioavailability.[14] Furthermore, self-emulsifying formulations typically provide greater drug loading capacity than other lipid-based formulations since the solubility of drugs with intermediate partition coefficients (logP value between 2 to 4) is usually low in natural oils but higher in co-solvents, amphiphilic surfactants, co-surfactants and their mixtures.[15,16]

Considering the intermediate logP (2 to 4) and low oral bioavailability of VEN, formulation of SMEDDS seems to be an appropriate technique for increasing solubility and bioavailability of the drug. Thus, the aim of study is formulation of SMEDDS of VEN and evaluation of its performance *in vivo*.

## MATERIALS AND METHODS

### Materials

Venlafaxine HCl was generously gifted by Wockhardt Pharmaceutical, Aurangabad. Caprol PEG 860 was gifted by Abitech Corporation, USA. Oleic acid, Tween 20, Tween 80, Aerosil 200, Propylene glycol, Polyethylene glycol 400 and were purchased from Research Lab, Mumbai.

### Methods

#### *Solubility Studies*

Saturation solubility of VEN was analyzed in various oils, surfactants, and cosurfactants. Excess amount of VEN was added to each screw capped glass vial containing 2 mL of solvent. The drug was mixed manually for half an hour followed by sonication for 15 to 20 min. The samples were then kept in orbital shaker at 25°C for 72 hr to reach equilibrium. The saturated solutions were then centrifuged at 3500 rpm for 20 min and supernatant was analyzed for drug content after appropriate dilution with methanol at 276 nm using UV spectrophotometry.[17-22]

### Screening of surfactants

The ability of various surfactants to emulsify selected oil was screened by the following method. Briefly, 15% w/v solution of surfactant in water was prepared. From this solution, 2.5 mL was taken in a test tube, it 10 µL of selected oil was added and then mixed by vortexing. The addition of oil was continued until solution became cloudy. The amount of oil required to produce turbidity was noted and the same procedure was repeated for all other surfactants.[23]

### Construction of phase diagrams

Pseudoternary phase diagrams for three-component system, oil, $S_{mix}$ (surfactant and cosurfactant in a fixed ratio) and water were constructed at room temperature by titration method. The microemulsion area was identified from the phase diagrams and the concentration range of each component of SMEDDS was optimized from this monophasic region. To plot the phase diagrams, oil and $S_{mix}$ (in a fixed ratio) were blended together in different weight proportions, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 (w/w) with magnetic stirring. The prepared blends were titrated very slowly with deionized water over the entire phase region. During each addition of water, the liquid phases were properly mixed to achieve equilibrium and observed for the phase change. The phase changes were noted as end point from which the region for the transparency (monophasic) and turbidity (biphasic) were identified. The pseudoternary phase diagrams of components, oil, surfactant:cosurfactant mixture and water were plotted using Triplot software. Different phase diagrams were constructed for different ratio of surfactant and cosurfactant in $S_{mix}$, viz. 1:2, 1:1, 2:1, 3:1. The monophasic microemulsion region in phase diagrams was identified and considering this region and solubility of drug, the proportions of components for formulating SMEDDS was selected.[24-26]

### Preparation of liquid SMEDDS

The procedure was used to prepare a series of SMEDDS formulations shown in Table 1. An accurately weighed Caprol PGE 860, Tween 20, and propylene glycol were added in a glass vial and mixed by vortexing. In the above formulation, VEN was

added and sonicated until it was fully dissolved. The formulation was kept at ambient temperature till further use.

## Characterization of SMEDDS

### *Determination of self-emulsification time*[27,28,25]

Self-emulsification efficiency of SMEDDS formulation was measured by the addition of formulation equivalent to one dose of VEN dropwise to 250 mL hydrochloric acid (0.1 N) with stirring at 100 rpm at 37±0.5°C. The time required for complete emulsification was noted visually.

## Dispersibility test and optical clarity

To simulate the *in vivo* post-administration fate of SMEDDS formulation upon dilution, a dispersibility test was conducted. The test was performed using USP dissolution apparatus II. SMEDDS formulation (1 mL) was added to dissolution vessel containing 500 mL of 0.1 N HCl at temperature 37 ± 0.5°C and 50 rpm. The dispersions were graded as follows:

Grade A: The nanoemulsion forms rapidly (within 1 min) with clear or bluish appearance.

Grade B: Rapidly forming emulsion with a bluish white appearance, and slightly less clear than A.

Grade C: Emulsification within 2 min resulting in fine milky emulsion.

Grade D: Emulsification after 2 min resulting in dull, grayish white and slightly oily appearance.

Grade E: An emulsion with minimal emulsification resulting in large oil globules

For the measurement of optical clarity, the dispersion produced after dispersibility test was analyzed for percent transmittance at 600 nm.[27-29,25] The formulation with visual grading A and percent transmittance 100% were selected for further studies.

## Thermodynamic stability studies[17-20]

These studies included exposure of formulation to thermal (both low and high) as well as mechanical stress and observing the effects on the self-emulsification ability and clarity of the SMEDDS formulation. The test was carried out in two parts:

## a) Alternate heating (40°C)/cooling (4°C) Cycle

It includes storage of formulations at each of these temperatures i.e, 4°C and 40°C alternately for not less than 48 hr for three cycles. Any kind of instability in the formulation was observed and change in dispersibility, self-emulsification time and optical clarity was noted. Only stable formulations were further tested for centrifugation.

## b) Centrifugation

It involved centrifuging the SMEDDS formulation for 30 min at 3500 rpm and evaluating whether they were stable based on change in dispersibility, self-emulsification time, and optical clarity. The formulations, which were thermodynamically stable, were only taken for further study.

**Table 1: Composition of liquid SMEDDS formulations with Caprol PGE 860, Tween 20 and Propylene Glycol.**

| Formulation code | Concentration (% w/w) of different components in SMEDDS | | % Drug |
|---|---|---|---|
| | Caprol PGE 860 | $S_{mix}$ | |
| S:Cos ratio 1:1 | | | |
| X1 | 9.41 | 84.70 | 5.89 |
| X2 | 18.82 | 75.29 | 5.89 |
| X3 | 28.00 | 65.88 | 5.89 |
| S:Cos ratio 1:2 | | | |
| X4 | 9.41 | 84.70 | 5.89 |
| X5 | 18.82 | 75.29 | 5.89 |
| X6 | 28.00 | 65.88 | 5.89 |
| S:Cos ratio 2:1 | | | |
| X7 | 9.41 | 84.70 | 5.89 |
| X8 | 18.82 | 75.29 | 5.89 |
| X9 | 28.00 | 65.88 | 5.89 |
| S:Cos ratio 3:1 | | | |
| X10 | 9.41 | 84.70 | 5.89 |
| X11 | 18.82 | 75.29 | 5.89 |
| X12 | 28.00 | 65.88 | 5.89 |

## Globule size analysis

Droplet size distribution of SMEDDS diluted with water was determined using Nanophox (NX0088, Symptec). SMEDDS samples (1 mL) were diluted by 250 mL of distilled water. Diluted sample was directly subjected to globule size measurement and all studies were made in triplicate.

## Preparation of solid SMEDDS using spray drying technique[17,30-33]

The optimized SMEDDS formulation was converted to solid SMEDDS (composition shown in Table 2) by spray drying technique. Briefly, Aerosil 200 was suspended in 50 mL water by overhead stirring. To this dispersion, liquid SMEDDS formulation was added with continuous stirring at room temperature until the uniform dispersion was obtained. The dispersion was then spray dried with a laboratory spray dryer (Labultima, LU 222 Advanced). The spray drying was performed at inlet temperature of 140°C, outlet temperature of 110°C and feed pump flow rate of 1 mL/min.

## Flow properties of solid SMEDDS

Micromeritic properties like bulk density, tapped density, angle of repose, Hausner ratio and Carr's index of solid SMEDDS were evaluated as per pharmacopoeial procedure.

## Scanning Electron Microscopy (SEM)[17,26,31,32]

SEM studies were performed to study the external morphology of spray dried solid SMEDDS formulation. The samples for SEM were prepared by mounting it on aluminium stub with the help of adhesive tape. A thin metal coating was imparted to the sample and the placed in sample chamber under vaccum (JEOL JSM-6360, Japan). The sample scanning was done at an acceleration voltage of 10 kV and photomicrographs were obtained at different magnification.

## Differential Scanning Calorimetry (DSC)[18,31,33]

A differential scanning calorimetry analysis was used to characterize physical state of drug in solid SMEDDS. The DSC patterns were recorded on a Mettler Toledo DSC Star system. In a nitrogen atmosphere, samples (2-4mg) were heated at 10°C/min rate in crimped aluminum pans at a temperature range of 30-400°C keeping empty pan as reference.

## X-ray Diffraction Studies[17,31,32,34]

Powder X-ray diffraction study was performed in the Brucker D 8 Advanced X-ray diffractometer. During analysis, Cu K 2 α ray was used at a voltage of 40 kV and a current of 25 mA. To obtain diffraction patterns for VEN, physical mixture and solid SMEDDS, the samples were scanned at 2θ angle from 10 to 60°.

## Percent Drug Content

The solid SMEDDS formulation equivalent to single dose was dissolved in 100 mL methanol using sonication. The resultant solution was filtered using Whatman filter paper and filtrate was appropriately diluted with methanol. The VEN content from the final solution was determined at 276 nm with a UV-spectrophotometer (UV 1700, Shimadzu) and drug content was calculated from a previously constructed standard curve in same solvent.[35]

## *In vitro* dissolution[7,36,37]

*In vitro* dissolution efficiency of developed formulation was tested using USP dissolution apparatus II (VDA-8D4, Veego Instruments). Considering the conditions of stomach, 500 mL of 0.1 N HCl was used as dissolution media. The peddle were set at 50 rpm and temperature was at 37±0.5°C. Liquid and solid SMEDDS formulationswere filled in hard gelatin capsule (size 00) before dissolution and sinkers were used during dissolution. At an interval of 5 min, an aliquot (5 mL) of each sample was collected and analyzed for VEN content using a UV-spectrophotometer at 274 nm. During each sample removal, an equivalent amount of fresh dissolution media was replaced to maintain the sink condition.[5,16]

## *In vivo* study of self-microemulsifying formulations[38-40]

For studying the *in vivo* performance of SMEDDS formulations, antidepressant and anti-anxiety activity of the developed formulations were evaluated using forced swim test and EPM

**Table 2: Data for composition of SMEDDS formulations.**

| Ingredients | Quantity |
|---|---|
| Composition of optimized Liquid SMEDDS (X1) | |
| Caprol PGE 860 (% w/w) | 9.41 |
| $S_{mix}$ (1:1) of Tween 20 and PG (%w/w) | 84.70 |
| Venlafaxine (%w/w) | 5.89 |
| Composition of solid SMEDDS (S1) | |
| SMEDDS (g) | 1 |
| AROSIL 200 (g) | 0.5 |
| Water (mL) | 50 |

maze test in Wistar strain rats. The studies were approved by institutional animal ethical committee and carried out as per CPCSEA guidelines.

### Animals

Wistar rats weighing 200-300 g were taken and divided into four groups of six rats each for both the experimental models. The standard environmental conditions such as, Light/dark cycle of 12 hr, temperature (24°C-26°C), and humidity (60%) were maintained and the animals were supplied with food and water as required. All animal cages were brought to experiment room, 2 hr before the experiment and formulations were given to the animals 1 hr before performance of the test. Group 1 was control without treatment, Group 2 was given plain SMEDDs formulations without drug, dispersion of VEN (16 mg/kg, oral) and VEN SMEDDs formulation (equivalent to 16 mg/Kg of Venlafaxine) was administered to group 3 and 4.

### a) *In vivo* anti-depressant activity by forced swim test in rats

In a forced swim test model, rats were exposed to stress by placing it in water filled container and behavioral changes (mobility and immobility) in rats with and without antidepressant formulation was compared. An acrylic transparent container of height 50 cm and diameter 20 cm was filled with water maintained at 25±2°C. The animal cages were shifted to testing environment 30 min before the procedure. The rats were preconditioned 24 hr prior to test by forcing them to swim in water filled container for 15 min. During test, after giving appropriate treatment as mentioned in the groups, each rat was transferred to cylinder filled with water and observed for 5 min using a video tracking system. If no movements are made by rat except the ones required for keeping its head above water, it was considered 'immobile', whereas the quick movements of swimming and struggle were coded as 'mobile'. The mean mobility and immobility time in seconds was noted. After the observations, the rat was removed from container, dried and placed under light for 30 min. After each session, the water in the container was replaced with fresh water so as to prevent the effect on the next rat.[6,41,42]

### b) *In vivo* anti-anxiety activity using EPM maze test

Anti-anxiety activity was evaluated using Elevated Plus maze (EPM) test in rats. EPM consists of two closed and two open arms in a plus shape. A 5 X 5 cm space, at the centre, connected these two arms. The closed and open arms were 50 cm in length and closed arms were of 10 cm height and width so that rats were able to pass through it. The maze was raised at 50 cm height above the ground. The EPM was kept in a separate quiet room with illumination of 50 lux. During the experiment, the rat was placed at the junction of close and open arm and facing the open arm. The number of entries made by the rat in each arm and average time spent in each open and close arm during 5 min of test was observed and tracked by video tracker. After each test, the maze was cleaned with acetic acid (1%) to prevent the effect on next rat.[42] Statistical treatment was given to collected data. Analysis of variance (ANOVA) followed by multiple comparison Bonferroni test was applied for the statistical comparison.

## RESULTS AND DISCUSSION

### Solubility study

At ambient temperature, self-micro emulsifying formulations should be a clear monophasic liquid. To enable the drug to be dispensed in aqueous phase after emulsification, it must be able to have good oil solubility. Among the various oils, Caprol PGE 860 (128±1.92 mg/mL) showed the highest solubility of VEN followed by Isopropyl myristate (26.01±0.232 mg/mL), Captex 500 (13.46±0.232 mg/mL), Captex 200P (12.62±1.20 mg/mL), and Captex 350 (9.31±0.90 mg/mL).

VEN was soluble in Tween 20 at 198±0.650 mg/mL and in Tween 80 at 24.6±0.650 mg/mL. Solubility of VEN in co-solvent was determined and found to be 32.00±1.22 mg/mL in propylene glycol and 48.00±1.22 mg/mL in polyethylene glycol 400. Based on this study, Caprol PGE 860 was selected for further study.

### Screening of surfactant

Surfactants play a major role in performance of SMEDDS. The formulation should immediately disperse upon dilution by gastrointestinal fluid just by gentle mixing. Considering the non toxicity of nonionic surfactants and their GRAS status (generally recognized as safe), Tween 20 and Tween 80 were screened. The results demonstrated greater emulsification ability of Tween 20 compared to Tween 80 for the oil Caprol PGE 860 and hence was selected for the formulation.

### Selection of co-solvents and construction of Pseudo Ternary Phase Diagrams

Upon addition into aqueous media, self-emulsifying systems create fine oil/water emulsions with only gentle agitation. In addition to reducing the interfacial energy and preventing coalescence, surfactants are preferentially adsorbed at the interface. By reducing the bending stress of the interface, co-solvent permits the interfacial film to bend over a variety of curvatures required to form microemulsions over a wide range of compositions while simultaneously increasing the solubility of lipophilic systems in aqueous solutions. Therefore, the selection of oil, surfactant, co-solvent, and the mixing ratio of oil to $S_{mix}$ are important factors in microemulsion formation.

From the solubility studies and screening of surfactants, Caprol PGE 860 (oil phase) was selected in combination with Tween 20 (surfactant) and propylene glycol (co-surfactant).

From Figure 1, the microemulsion region is evident for Caprol PGE 860, Tween 20 and PG system and shown as shaded one

phase region. At oil:$S_{mix}$ ratio of 1:9, clear one phase region was obtained even after infinite dilution. When S: Cos ratio was 1:1, the O/W microemulsion region was maximum. When the ratio was changed to 3:1 to 1:2 (decrease in surfactant concentration), the shaded one phase region was found to be slightly decreased.

## Formulation of liquid SMEDDS formulations with drug

On the basis of preliminary studies, a series of liquid SMEDDS formulations with varying concentration of oil, surfactant and cosolvent were selected from ternary phase diagram. The following points were considered while selecting the formulations from one phase region of pseudoternary phase diagram.

## Dose of drug

The quantity and composition of SMEDDS should be sufficient to solubilize the unit dose of VEN (25 mg). The addition of water soluble surfactant, Tween 20 (HLB>12) and co-surfactant (propylene glycol) would help in solubilizing the drug.

## Region of microemulsion at infinite dilution

Mixtures of oils and $S_{mix}$ showing clear one phase region till infinite dilution (as evident in the pseudoternary phase diagrams) were considered for Self-microemulsifying formulations since these mixtures upon dilution in intestine would result into clear microemulsion with globule size of less than 100 nm which might help in improving the bioavailability of the drug.

## Precipitation of the drug after dilution

In SMEDDS formulations, concentration of oil was restricted to 10% to 30% w/w because with decreased amount of oil content and more $S_{mix}$ in the formulation, the oil droplets become less susceptible to digestion thus lowering the risk of precipitation of the drug. Although, the addition of surfactant and co-surfactant above 40% might result into very fine dispersions, formulations with very high amounts of surfactant and co-surfactant might also lead to precipitation of the drug. Hence, the optimum concentration of surfactant and co-solvent was needed so as to obtain a fine dispersion without precipitation of the drug.

## Evaluation of Liquid SMEDDS Formulations

### *Determination of emulsification time*

The efficiency of SMEDDS can be assessed by visually evaluating the rate of emulsification of the system after addition to large quantity of water. An ideal SMEDDS system should disperse quickly and completely under mild agitation to aid absorption of the drug in the gastrointestinal tract. Results in Table 3 indicate the effect of $S_{mix}$ concentration and S:Cos ratio on the emulsification time of the formulations. As $S_{mix}$ concentration was increased in the formulation, the self emulsification time decreased. This might be attributed to quick solubilization of co-solvent in aqueous phase leading to immediate dispersion and emulsification of the system.

## Dispersibility test and Optical clarity

When SMEDDS containing hydrophilic surfactants and co-solvents are diluted with water, the surfactant and co-solvent phase rapidly separates from lipid phase. This might cause precipitation of the drug or coalescence of oil globule if the surfactant and co-solvent are not in optimum concentration in the formulation. Therefore, all the formulations upon dilution were visually observed and graded for their transparency. Similarly, the percent transmittance of diluted formulations was also noted at 600 nm.

The formulation with higher concentration of $S_{mix}$ obtained transparent emulsions with grade A upon dilution. Thus, increase in the surfactant concentration in the formulation led to decrease in droplet size of resulting emulsion resulting into finer emulsions.

## Thermodynamic stability studies

In all SMEDDS formulations, there was no significant change in the self-emulsifying performance during thermodynamic stability study. There was no precipitation in formulation with or without dilution during the period of study. Thus, all the formulations were found to be thermodynamically stable. Considering the performance, X1 formulation was optimized for further study.

## Globule size analysis

The size of the globules after emulsification of SMEDDS is an important parameter for enhancing the bioavailability of drugs. The smaller globule size provides large interfacial area for drug absorption. The globule size of microemulsion is expected to be below 100 nm.

Formulation X1 showed an average droplet size of 21.03 nm ($X_{90}$) indicating desirable size with uniform size distribution (PDI 0.22).

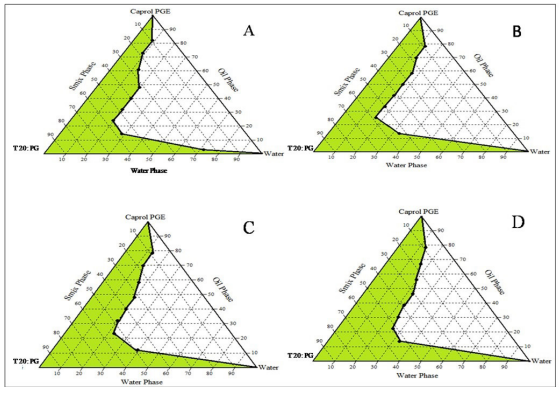


**Figure 1:** Pseudo ternary phase diagram of system with the following components: Caprol PGE 860 (oil), Tween 20:Propylene glycol, A-1:1, B-1:2, C-2:1, D-3:1 ($S_{mix}$).

**Table 3: Data of Evaluation of SMEDDS formulation.**

| Form code | Disp. Time * (sec) | % T* | Visual grade | Thermo-dynamic stability |
|---|---|---|---|---|
| S:co S ratio 1:1 | | | | |
| X1 | 24±1.00 | 100±0.00 | A | Stable |
| X2 | 26±0.17 | 99.90±0.29 | A | Stable |
| X3 | 17±0.65 | 82.8±0.83 | B | Stable |
| S:co S ratio 1:2 | | | | |
| X4 | 25±0.58 | 100±1.0 | A | Stable |
| X5 | 28±0.50 | 100±0.41 | A | Stable |
| X6 | 29±1.00 | 85±0.75 | B | Stable |
| S:co S ratio 2:1 | | | | |
| X7 | 25±0.11 | 100±0.84 | A | Stable |
| X8 | 28±1.06 | 97.7±1.02 | A | Stable |
| X9 | 30±1.12 | 81.20±0.95 | B | Stable |
| S:co S ratio 3:1 | | | | |
| X10 | 26±0.17 | 100±1.00 | A | Stable |
| X11 | 27±0.50 | 100±1.48 | A | Stable |
| X12 | 27±0.27 | 84.00±0.83 | B | Stable |

## Evaluation of Solid SMEDDS

### *Flow properties*

The flow properties play an important role in filling process of the solid SMEDDS in capsules. The solid SMEDDS should have good flow properties in order to dispense it in a suitable form. The flow properties of formulation were determined in terms of bulk density (0.2083±0.0026), tapped density (0.2173±0.0012), Hausner's ratio (1.04±0.015), Carr's index (4.18±0.98), angle of repose (22.21±1.23°), and found to be acceptable.

## Scanning Electron Microscopy (SEM)

Figure 2 shows scanning electron micrographs of VEN, Aerosil 200 powder, and solid SMEDDS formulation. VEN (Figure 2A and B) appeared crystalline and elongated in shape. As shown in Figure 2 C and D, the Aerosil 200 powder has a rough surface and highly porous particles arranged in agglomerates. From the SEM image, the Solid SMEDDS formulation showed spherical particles with well-separated dense surfaces (Figure 2 E and F).

## Differential Scanning Calorimetry (DSC)

DSC curves of pure VEN and solid SMEDDS of VEN are shown in Figure 3. Pure VEN showed a sharp endothermic peak at temperature 212°C indicating melting of VEN crystalline structure. Absence of endothermic peak in solid SMEDDS could be due to complete solubilization of VEN in oil phase of formulation.

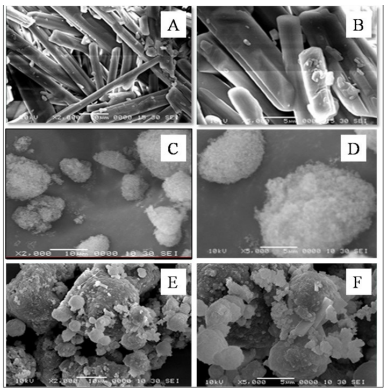


**Figure 2:** Scanning electron micrographs of pure drug (A: X2000, B: X5000), Physical mixture at (C: X2000, D: X5000) and of Solid SMEDDS at (E: X2000, F: X5000).

## X-ray Diffraction Studies

The internal physical state of VEN in solid SMEDDS was further confirmed by X-ray powder diffractograms shown in Figure 4. Curve A shows sharp, intense peaks corresponding to the crystalline structure of VEN. Due to the dilution by Aerosil 200, very small peaks appeared in the physical mixture of VEN and Aerosil 200, however, characteristic peak of drug were observed indicating crystalline nature of drug (curve B). No obvious peaks representing crystals of VEN were seen for the solid SMEDDS confirming the complete solubilization of drug in oil (curve C).

## Percent Drug Content

The drug content was found in the range of 95-100%.

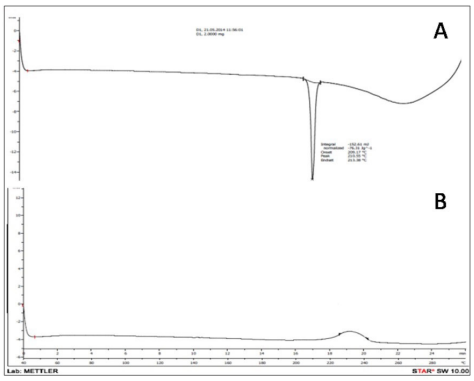


**Figure 3:** DSC Spectra of pure drug VEN (A) and Solid SMEDDS (B).

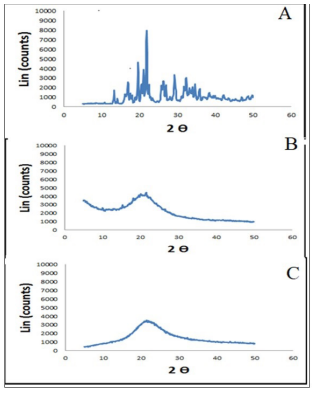


**Figure 4:** X-ray diffraction pattern of curve A) VEN (pure drug) Curve B) Physical mixture and Curve C) solid SMEDDS.

## Drug release studies

*In vitro* dissolution study (Figure 5) indicated more than 50% of drug release in 15 min and 90% of VEN release within 30 min of dissolution (Figure 5), whereas only 40.3% of the drug was released from the pure drug solution after 30 min. This indicated improvement in dissolution by SMEDDS formulation. The developed formulation dispersed almost instantaneously indicating the high self-emulsification efficiency.

## *In vivo* anti-depressant activity of liquid SMEDDS

### *Forced swim test in rats*

*In vivo* anti-depressant activity of optimized SMEDDS formulation was studied and compared with that of oral dispersion of VEN and control group. One way analysis of variance suggested significant effect of treatment groups compared to control with no treatment and solvent control or placebo ($p<0.001$). There was increase in mobility and decrease in immobility and climbing in the treatment groups. The depression is characterized by psychomotor impairment resulting in immobility behavior in animal model. Antidepressant treatment stimulates the pshychomotor activities, reduces immobility and increases mobility. The multiple comparison Bonferroni test revealed significant antidepressant activity of SMEDDS formulation

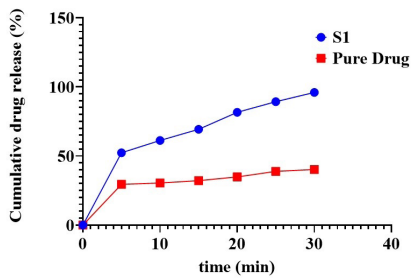


**Figure 5:** *In vitro* drug release profile of VEN and optimized S-SMEDDS formulation (S1).

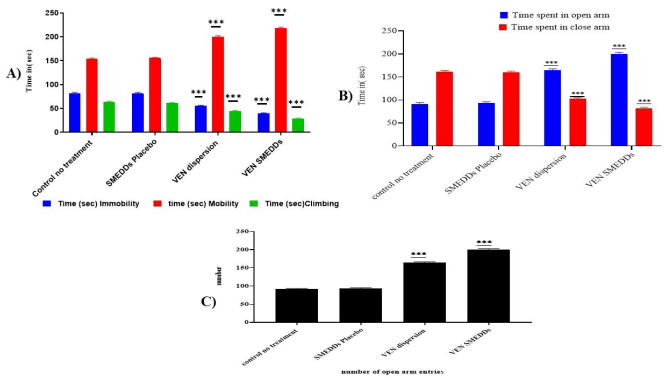


**Figure 6:** Effect of various formulation of VEN on immobility, mobility and climbing in Forced swim test in rats (A), Effect on time spent in open arm and time spent in closed arm (B), Effect on open arm entry (C).

in comparison to VEN dispersion ($p<0.001$). This indicated improved efficacy of SMEDDS formulation over conventional dispersion.

## Elevated plus maze

In elevated plus maze, an open arm entry and the amount of time spent in an open arm have traditionally been used as indices of anxiety. A cluster of behaviors referred to as "risk assessment" is comprised of entry latency, and non-exploratory behavior. These responses (such as scanning, stretching, and laying on the back) are usually observed in potentially dangerous situations and are highly sensitive to the effects of anxiety-modulating drugs.

From Figure 6 A, it is evident that treatment groups were significantly effective in time spent in open arm (**$p<0.001$), time spent in closed arm (**$p<0.001$)(Figure 6B) and number of open arm entries (**$p<0.001$) (Figure 6C) throughout the elevated plus-maze model in rat. The higher number of open arm entries and more average time spent in open arm indicated low anxiety behavior whereas close arm entries indicated high anxiety. Thus, the data confirmed that VEN SMEDDS formulation was effective in controlling the anxiety over oral dispersion.

## CONCLUSION

An optimized SMEDDS formulation of Venlafaxine consisting of Caprol PGE 860 (9.41% w/w), Tween 20 (42.35% w/w) and PG (42.35% w/w) was successfully developed with globule size of 21 nm. Spray drying of liquid SMEDDS formulation to solid SMEDDS was successful using solid carrier Aerosil 200. The optimized solid Self-microemulsifying formulation (9.41% oil, 42.35% surfactant and 42.35% co-surfactant and liquid SMEDDS: carrier ratio of 1:0.5) was found to release more than 90% of drug in 30 min. *In vivo* antidepressant and anti-anxiety activity in rats demonstrated improved performance of developed formulation compared to oral standard. Thus, our study revealed that the self-microemulsifying formulation can be a suitable formulation approach in improving the efficacy of VEN.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**SMEDDS:** Self-Microemulsifying Drug Delivery System; **SEM:** Scanning Electron Microscopy; **DSC:** Differential Scanning Calorimetry; **EPM:** Elevated Plus maze.

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