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# Preparation And In Vivo Evaluation Of Smedds Containing Nevirapine For Bioavailability Improvement

V. Vijay Kumar*[1, 2], J. Raju[1], D.V. R. N. Bhikshapathi[3], K. Naga Laxmi[3]

*Corresponding author:

V. Vijay Kumar

[1]Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500072, Telangana, India.

[2]Vaagdevi College of Pharmacy, Ramnagar, Hanamkonda-506001, Telangana, India.

[3]Vijaya College of Pharmacy, Hayath Nagar, Hyderabad-501511, Telangana, India.

## Abstract

Nevirapine has been formulated in lipid-based system, a Self Emulsifying Drug Delivery System (SEDDS) to target the drug to lymphoid organs where HIV-1 virus resides in large population. Nevirapine SEDDS were formulated for enhancement of solubility, dissolution rate and oral bioavailability of model drug Nevirapine. Fourteen formulations were prepared using different oils, surfactants and co-surfactants. A pseudo ternary phase diagram was constructed to identify the self-micro emulsification region. Further, the resultant formulations were investigated for clarity, phase separation, drug content, % transmittance, globule size, freeze-thaw stability and in vitro dissolution studies. On the basis of dissolution profile and other above mentioned studies, F4 was found to be the best formulation of Nevirapine SEDDS which contains Capryol 90 (Oil), Tween 80 and PEG 600 as surfactant co-surfactant respectively. In vivo studies revealed that the oral bioavailability of Nevirapine from SEDDS was 2-fold higher compared to that of pure Nevirapine suspension in rats, suggesting a significant increase in oral bioavailability of Nevirapine from SEDDS formulation. The higher bioavailability might be due to the enhanced solubility of Nevirapine by SEDDS formulation.

**Keywords:** Nevirapine, SEDDS, Capryol 90, Pharmacokinetics, Bioavailability studies,

## Introduction

As oral route for drug administration is most commonly used among all the routes of administration due to its convenience, non-invasiveness and cost effectiveness it become necessary that drug should have some aqueous as well as some lipid solubility for their absorption [1]. Lipid-based formulation approaches, particularly the self-microemulsifying drug delivery system (SMEDDS), are well known for their potential as alternative strategies for delivery of hydrophobic drugs [2], which are associated with poor water solubility and low oral bioavailability [3-5]. SMEDDS formulations are isotropic mixtures of oil, a surfactant, a co-surfactant, and a drug. The basic principle of this system is its ability to form fine oil-in-water (o/w) micro emulsions under gentle agitation following dilution by aqueous phases [6]. This spontaneous formation of an emulsion in the gastrointestinal tract presents the drug in a solubilized form, and the small size of the formed droplet provides a large interfacial surface area for drug absorption [7]. Apart from solubilization, the presence of lipid in the formulation further helps improve bioavailability by affecting the drug absorption. Selection of a suitable self- emulsifying formulation depends upon the assessment of the solubility of the drug in various components, the area of the self-emulsifying region as obtained in the phase diagram, and the droplet size distribution of the resultant emulsion following self-emulsification [8]. Nevirapine is a non-nucleoside reverse transcriptase inhibitor of human immunodeficiency virus, type 1 (HIV-1). The drug is practically insoluble in water (0.1 mg/ml), belongs to Class II as per Biopharmaceutical Classification System[9] with a log octanol-water partition coefficient (log P) of 2.5 and a pKa of 2.8[10]. HIV enters the human host via mucosal surfaces and is subsequently disseminated throughout the lymphatic tissues, a major reservoir of virus throughout the course of infection [11, 12].

## Materials and methods

### Materials

Viramune (Nevirapine 200mg) tablets purchased from Boehringer Ingelheim, Mumbai. Nevirapine pure drug, Lauroglycol, Labrasol was generous gifts from Aurobindo Pharma Limited, Hyderabad, India. Castor oil, Capryol 90, Miglynyl 812, Captex 355 and Olive oil were obtained from Granules India limited, Hyderabad. Kolliphor HS 15, Kolliphor RH 40, Labrasol, Lauroglycol, Labrafil M 2125, Labrafil M 1944CS were gifted from BASF, Mumbai. Tween 80, Propylene glycol, PEG 400 and PEG 600 were obtained from SDFCL, Mumbai. All other chemicals used were of analytical grade.

## Methods

### Solubility studies

It was carried out to determine solubility measurements of Nevirapine according to the published method [13] The solubility

study was used to find out the suitable oil, surfactant and co-surfactant that possess good solubilizing capacity for Nevirapine. An excess amount (250 mg) of Nevirapine was added into 1 ml of each excipient (Oils – Captex-355, Capryol-90, Castor oil, Miglynyl 812, Oliec acid, )(surfactants – Kolliphor HS 15, Kolliphor RH 40, Kolliphor PS 80, Kolliphor ELP, Kolliphor EL, Labrasol, Tween-20, Tween-80, Cremophor RH 40, Transcutol-P, Labrafac, Labrafil M 2125, Labrafil M 1944cs, Capmul MCM) (co-surfactants - PEG 400, PEG 600, Propylene glycol etc) and kept in mechanical shaker for 24 hrs and centrifuged at 2500 rpm for 20 min using a centrifuge. The supernatant was appropriately diluted with methanol, and UV absorbance was measured at 264 nm. Concentration of dissolved drug was determined spectrophotometrically.

## Pseudoternery phase diagram

Pseudo ternary phase diagram is used to map the optimal composition range for three key excipients according to the resulting droplet size following self emulsification, stability upon dilution and viscosity. On the basis of the solubility studies of drug in oil, surfactants and co-surfactants were used for construction of phase diagram. Surfactant and co-surfactant (Smix) in each group were mixed in different volume ratio (1:1, 2:1, 3:1).Oil and surfactant/co-surfactant mixture (Smix) were mixed thoroughly in different volume ratios 1:9 to 9:1 (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, and 1:1) and (9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, and 2:1) w/w. The mixture of oil, surfactant and co-surfactant at certain ratios were titrated with water by drop wise addition under gentle agitation. Deionized water was used as diluting medium and added into the formulation. The proper ratio one excipient to another in the SMEDDS formulation was analysed. Pseudo ternary plots were constructed using Chemix software.

## Development of SMEDDS formulation

A series of SMEDDS formulation for Nevirapine were prepared based on solubility studies, pseudo ternary phase diagram and visual observation. Here, Capryol 90 (Capryol PGMC) was used oil phase and Tween 80 and PEG 600 were used as surfactant and co-surfactant respectively. The composition was given in the Table. In brief, Nevirapine (200 mg) was added in accurately weighed amount of oil into screw – capped glass vial and heated in a water bath at 40 C. The surfactant and co-surfactant were added to the oil mixture using positive displacement pipette and stirred with magnetic bar. The formulation was further sonicated for 15mins and stored at room temperature until its use in subsequent studies.

**Table 1: Formulation trials of liquid SMEDDS**

| Smix (Surfactant: Co-surfactant) | Oil:Smix | Formulation Code | Oil (Capryol 90 ) (ml) | Surfactant (Tween 80) (ml) | Co-surfactant (PEG 600) (ml) |
|---|---|---|---|---|---|
| 1:1 | 1:9 | F1 | 0.400 | 1.80 | 1.80 |
| | 1:8 | F2 | 0.444 | 1.776 | 1.776 |
| | 1:7 | F3 | 0.5 | 1.750 | 1.750 |
| | 1:6 | F4 | 0.541 | 1.713 | 1.713 |
| 2:1 | 1:9 | F5 | 0.400 | 2.400 | 1.200 |
| | 1:8 | F6 | 0.444 | 2.368 | 1.184 |
| | 1:7 | F7 | 0.500 | 2.332 | 1.166 |
| | 1:6 | F8 | 0.571 | 2.284 | 1.142 |
| | 1:5 | F9 | 0.666 | 2.220 | 1.11 |
| 3:1 | 1:9 | F10 | 0.4 | 2.7 | 0.9 |
| | 1:8 | F11 | 0.444 | 2.664 | 0.888 |
| | 1:7 | F12 | 0.5 | 2.625 | 0.875 |
| | 1:6 | F13 | 0.571 | 2.569 | 0.856 |
| | 1:5 | F14 | 0.666 | 2.497 | 0.832 |

## Freeze thawing

Freeze thawing was employed to evaluate the stability of formulations. The formulations were subjected to 3 to 4 freeze-thaw cycles, which included freezing at – 4 C for 24 hours followed by thawing at 40 C for 24 hours. Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies.

% Transmittance

% Transmittance of Nevirapine SMEDDS was measured by U.V spectroscopy at wavelength of 600 to 660nm. A graph for %particle range vs. formulations was plotted.

## Determination of drug content

SMEDDS equivalent to 200mg of Nevirapine were weighed accurately and dissolved in 100ml of 0.1N HCL. The solution was filtered, diluted suitable and drug content was analyzed at $\lambda_{max}$ 264 nm against blank by UV spectrometer. The actual drug content was calculated using the following equation as follows:

$$\% \text{ Drug content} = \frac{\text{Actual amount of drug in SMEDDS}}{\text{Theoretical amount of drug in SMEDDS}} \times 100$$

## *In-vitro* dissolution studies

The release of drug from liquid SMEDDS formulations and pure drug was determined using a US Pharmacopoeia Type II dissolution apparatus. The liquid SMEDDS formulations were directly placed into the medium [14]. The dissolution media is 0.1N HCL, and temperature of the dissolution medium was maintained at $37^0$C operated at 75 rpm. An aliquot of 5 ml was withdrawn at predetermined intervals 2, 5, 10, 15, 20, 25, 30, 45, and 60 mins and filtered through 0.45-µm pore size membrane filters. The removed volume was replaced each time with 5 ml of fresh medium. The concentrations were assayed spectrophotometrically at 264nm.

## Characterization of SEDDS

## Drug-excipient compatibility studies

## Fourier transform infrared spectroscopy (FTIR)

The IR spectra of pure drug, excipients and optimized formulations were recorded using FT-IR (Shimadzu 8400-S) with diffuse reflectance principle. Sample preparation involved, drying of potassium bromide (KBr), drug and excipients in the oven to get rid of any moisture content then mixing the sample with KBr by triturating in glass mortar. Finally preparing of pellet and placing in the sample holder. The spectrum was scanned over a frequency range 4000 – 400 $cm^{-1}$ [15].

## Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Accurately weighed samples were placed on aluminium plate, sealed with aluminium lids and heated at a constant rate of 5ºC /min, over a temperature range of 0 to 250ºC [16].

## Determination of droplet size

The average droplet size of Nevirapine SMEDDS formulations were determined by Photon correlation spectroscopy (Malvern Instrument UK) able to measure sizes between 10 and 5000 nm. The selected formulations were diluted with deionized water and placed in an electrophoretic cell for measurement [17].

## Determination of zeta potential

The emulsion stability is directly related to the magnitude of the surface charge. In conventional SMEDDS, the charge on an oil droplet is negative because of the presence of free fatty acids. The zeta potential of the diluted SMEDDS formulation was measured using a zeta meter system. The SMEDDS were diluted with a ratio 1:2500 (v/v) with distilled water and mixed with magnetic stirrer. Zeta-potential of the resulting micro emulsion was determined using a Malvern Zetasizer [18].

## Scanning electron microscopy

The surface and shape characteristics of pellets were determined by scanning electron microscopy (SEM) (HITACHI, S-3700N). Photographs were taken and recorded at suitable magnification.

## Stability studies

The SMEDDS formulations were put into empty hard gelatin capsules and subjected to stability studies at 40 C/75% RH. Samples were charged in stability chambers (Thermo lab, Mumbai, India) with humidity and temperature control. They were withdrawn at specified Accelerated conditions for 6months. Dissolution studies and drug content of the capsules was analyzed using a previously developed and validated stability-indicating UV method.

## In vivo bioavailability studies

## Animals

Healthy Wistar rats were (Weighing 150-180 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature $25^0$C, Relative Humidity 45% and 12 h alternate light and dark cycle) with 100% fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and water ad libitum. The protocol of this study was approved by the institutional animal ethics committee.

## Study Design

Healthy Wistar rats were divided in to two groups at random containing six animals each. The rats were fasted for 24 hours prior to the experiments. After 4 hours of dosing, foods were reoffered. First group was administered with pure Nevirapine (as such) made suspension with 0.5% methocel and second group was administered liquid SMEDDS diluted in 0.5% methocel by oral route at a dose of 200mg equivalent to animal body weight. Then, 500 µL blood samples were collected from the retro-orbital vein using a heparinized needle (18-20 size) at 0, 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00,16.00 and 24.00 hrs post dose and transferred into Eppendorf tubes containing heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5min to 10 minutes and stored frozen at 20 C until analysis.

## Determination of Nevirapine in rat plasma by HPLC method

Determination of Nevirapine and internal standard Carbamazepine by high performance liquid chromatography using a RP-C18 chromatographic column, Phenomenex Kinetex (150 mm 4.6 mm i.d) and a mobile phase consisting of 15 mM aqueous phosphate buffer: acetonitrile (65:35 % v/v) at a flow rate 1.0 mL/min and the wavelength detection was 283 nm. The retention time for Nevirapine and internal standard Carbamazepine was found to be 5.1 min and 6.2 min respectively [19].

## Pharmacokinetic data analysis for liquid SMEDDS and pure drug suspension

The pharmacokinetic parameters employed to evaluate were maximum plasma concentration ($C_{max}$), time to attain $C_{max}$ i.e., $T_{max}$ and $t_{½}$ values, area under plasma concentration–time curve from zero to the last sampling time ($AUC_{0-t}$), area under plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$). $AUC_{0-t}$ was calculated by the linear trapezoidal rule and $AUC_{0-\infty}$ from the following formula.

$AUC_{0-\infty} = AUC_{0-t} + C_t / K_E$

# Results and discussion

## Solubility studies

The Nevirapine pure drug solubility in water was found to be 7.04 mg/ml. The solubility of the Nevirapine pure drug was tested in different oil phases and maximum solubility was found in Capryol 90 as 32.23 mg/ml (Figure 1). The solubility was tested in different surfactants and co-surfactants, maximum solubility was found in Tween 80 and PEG 600 as 34.56 mg/ml and 40.46 mg/ml was in PEG 600 respectively (Figure 2 & 3). Capryol 90, Tween 80 and PEG 600 were used for the formulation of Nevirapine SEDDS.

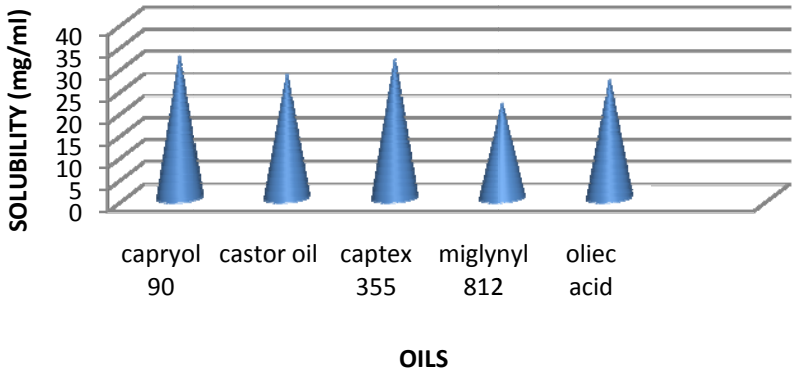


Figure 1: Solubility studies of Nevirapine in oils

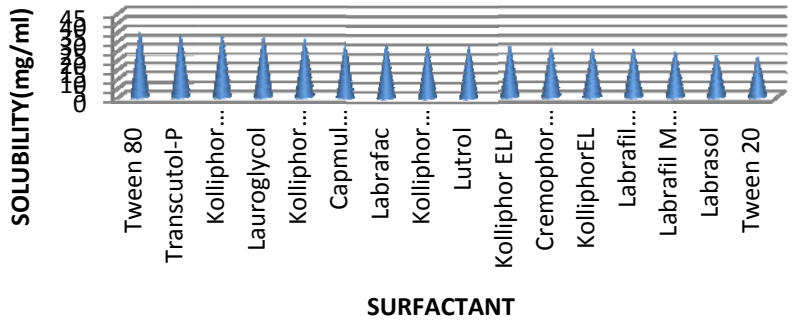


Figure 2: Solubility studies of Nevirapine in surfactants

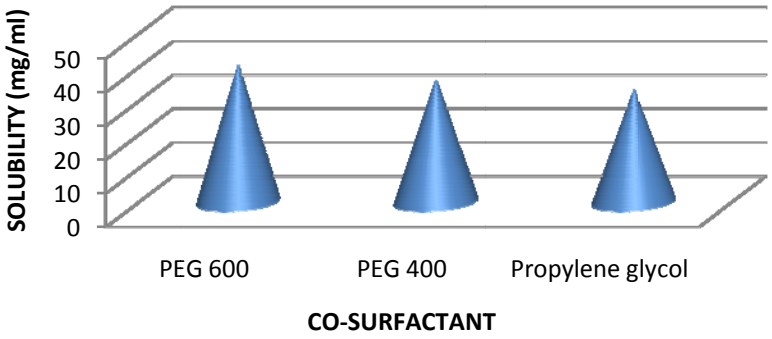


**Figure 3: Solubility studies of Nevirapine in co-surfactants**

## Pseudo ternary phase diagram

From the solubility studies, Capryol 90, Tween 80, and PEG 600 were selected as oil, surfactant and co-surfactant respectively. From the phase diagram shown in Figure, it was observed that self emulsifying region was enhanced with increasing concentrations of surfactant and co-surfactant with oil. Efficiency of self-emulsification was good when the surfactant concentration increased

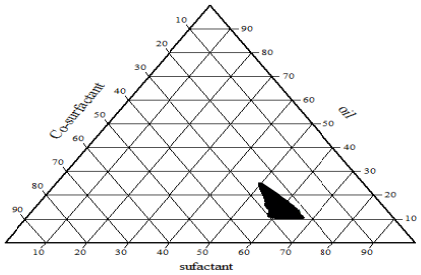


**Figure 4: Ternary phase diagram of Capryol 90, Tween 80, and PEG 600**

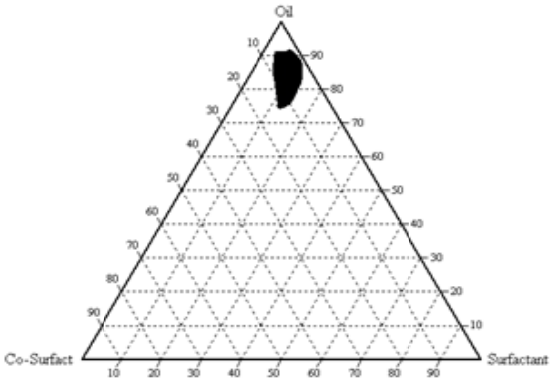


**Figure 5: Ternary phase diagram of castor oil, Tween 80 and PEG 600**

## Preparation of Nevirapine SMEDDS

SMEDDS of Nevirapine were prepared by using Capryol 90 (oil), Tween 80 (surfactant), and PEG 600 (co-surfactant). In the present study, fourteen formulations were prepared and their complete composition was shown in Table. All the formulations prepared were found to be clear and transparent. Pictorial representations of different formulations were shown in Figure.

**Figure 6: Different Nevirapine SMEDDS formulations**

## Freeze thaw method

In thermodynamic stability study, no phase separation and no change of temperature variations on prepared formulations were observed. There was no change in the visual description of samples after centrifugation freeze-thaw cycles.

## % Transmittance measurement & Drug content

The clarity of micro emulsions was checked by transparency, measured in terms of transmittance (%T). SMEDDS forms o/w microemulsion since water is external phase Formulation F4 has % transmittance value greater than 99%.These results indicate the

high clarity of microemulsion. In case of other systems %T values were less than 99% suggesting less clarity of microemulsions. This may be due to greater particle size of the formulation. Due to higher particle size, oil globules may reduce the transparency of microemulsion and thereby values of %T. The results of %T are as shown in Table 2. The drug content of the prepared SMEDDS was found to be in the range of 91.30 - 98.63%. Maximum % drug content i.e. 98.63% was found in the formulation F4. The results of visual observation % Transmittance and drug content were shown in Table 2.

**Table 2: % Transmittance of different formulations**

| S. No. | Formulation Code | % Drug content | % Transmittance |
|---|---|---|---|
| 1 | F1 | 94.36 | 99.959 |
| 2 | F2 | 94.21 | 99.387 |
| 3 | F3 | 92.53 | 99.867 |
| 4 | F4 | 98.63 | 99.971 |
| 5 | F5 | 91.95 | 99.678 |
| 6 | F6 | 92.20 | 99.225 |
| 7 | F7 | 94.67 | 99.619 |
| 8 | F8 | 93.75 | 99.536 |
| 9 | F9 | 91.60 | 99.951 |
| 10 | F10 | 92.64 | 99.541 |
| 11 | F11 | 94.53 | 99.954 |
| 12 | F12 | 93.26 | 99.052 |
| 13 | F13 | 91.30 | 99.631 |
| 14 | F14 | 94.59 | 99.303 |

## *In-vitro* dissolution studies of SMEDDS

The results of in vitro dissolution comparisons of SMEDDS formulations are summarized in Table 3, 4 and Figure 7, 8. The faster dissolution from SMEDDS may be attributed to the fact that in this formulation, the drug is a solubilized form and upon exposure to dissolution medium results in small droplet that can dissolve rapidly in the dissolution medium. The release from liquid SMEDDS formulation F4 was faster than other SMEDDS formulations and pure drug substance indicating influence of droplet size on the rate of drug dissolution.

Table 3: *in vitro* Cumulative % Drug Release for Formulations (F1-F8), pure drug and innovator

| Time (mins) | Cumulative % drug release | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pure drug | Innovator product (Viramune 200mg) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
| 0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| 2 | 2.21±0.12 | 40.03±0.51 | 51.68±0.32 | 37.62±0.55 | 38.09±0.25 | 53.01±0.21 | 31.45±0.56 | 35.56±0.51 | 50.62±0.42 | 28.03±0.28 |
| 5 | 6.34±0.42 | 65.21±0.42 | 63.94±0.56 | 53.43±0.75 | 50.78±0.15 | 68.31±0.54 | 52.34±0.52 | 48.91±0.55 | 63.75±0.12 | 46.71±0.24 |
| 10 | 10.91±0.24 | 74.63±0.32 | 75.50±0.32 | 64.22±0.32 | 65.05±0.41 | 80.63±0.52 | 63.73±0.75 | 64.24±0.53 | 78.92±0.53 | 57.82±0.26 |
| 15 | 14.78±0.56 | 77.99±0.22 | 76.43±0.24 | 70.84±0.52 | 74.82±0.24 | 84.62±0.58 | 66.36±0.32 | 70.89±0.23 | 80.34±0.75 | 60.01±0.52 |
| 20 | 17.43±0.52 | 78.58±0.43 | 77.60±0.52 | 72.38±0.42 | 79.36±0.32 | 86.55±0.69 | 67.13±0.25 | 71.04±0.52 | 81.68±0.59 | 62.34±0.24 |
| 25 | 20.11±0.35 | 79.39±0.77 | 77.95±0.15 | 72.96±0.36 | 80.21±0.54 | 88.31±0.55 | 67.82±0.24 | 72.01±0.23 | 82.74±0.24 | 64.78±0.32 |
| 30 | 23.32±0.52 | 80.57±0.45 | 78.43±0.32 | 73.84±0.41 | 81.36±0.55 | 88.92±0.72 | 68.81±0.21 | 72.33±0.21 | 82.99±0.53 | 65.52±0.74 |
| 45 | 31.57±0.75 | 81.24±0.32 | 79.05±0.16 | 75.06±0.35 | 81.75±0.51 | 91.25±0.52 | 70.08±0.32 | 73.68±0.56 | 83.61±0.31 | 65.09±0.12 |
| 60 | 32.84±0.55 | 81.76±0.28 | 81.26±0.15 | 75.95±0.52 | 82.64±0.21 | 93.64±0.42 | 72.22±0.21 | 74.53±0.62 | 84.14±0.54 | 67.34±0.23 |

Table 4: *in vitro* Cumulative % Drug Release for Formulations (F9-F14), pure drug and innovator

| Time (mins) | Cumulative % drug release | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pure drug | Innovator product (Viramune 200mg) | F9 | F10 | F11 | F12 | F13 | F14 |
| 0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| 2 | 2.21±0.12 | 40.03±0.51 | 53.02±0.25 | 41.24±0.42 | 34.42±0.23 | 31.33±0.25 | 42.63±0.42 | 53.34±0.42 |
| 5 | 6.34±0.42 | 65.21±0.42 | 68.79±0.36 | 61.82±0.26 | 50.61±0.29 | 69.06±0.54 | 53.34±0.54 | 64.32±0.85 |
| 10 | 10.91±0.24 | 74.63±0.32 | 73.54±0.29 | 71.54±0.28 | 61.33±0.53 | 77.85±0.26 | 66.46±0.85 | 73.65±0.68 |
| 15 | 14.78±0.56 | 77.99±0.22 | 74.61±0.58 | 72.93±0.65 | 65.54±0.42 | 79.62±0.19 | 71.97±0.52 | 75.83±0.52 |
| 20 | 17.43±0.52 | 78.58±0.43 | 75.02±0.27 | 73.08±0.32 | 66.74±0.29 | 81.13±0.42 | 72.24±0.54 | 76.15±0.28 |
| 25 | 20.11±0.35 | 79.39±0.77 | 75.94±0.52 | 73.64±0.85 | 66.91±0.79 | 81.95±0.75 | 72.86±0.32 | 76.84±0.54 |
| 30 | 23.32±0.52 | 80.57±0.45 | 76.81±0.56 | 75.17±0.45 | 67.35±0.56 | 82.22±0.42 | 74.34±0.33 | 77.68±0.41 |
| 45 | 31.57±0.75 | 81.24±0.32 | 78.12±0.75 | 76.02±0.12 | 68.04±0.18 | 83.67±0.35 | 75.91±0.18 | 78.92±0.52 |
| 60 | 32.84±0.55 | 81.76±0.28 | 80.31±0.32 | 77.13±0.21 | 68.72±0.86 | 84.85±0.62 | 76.37±0.15 | 79.31±0.75 |



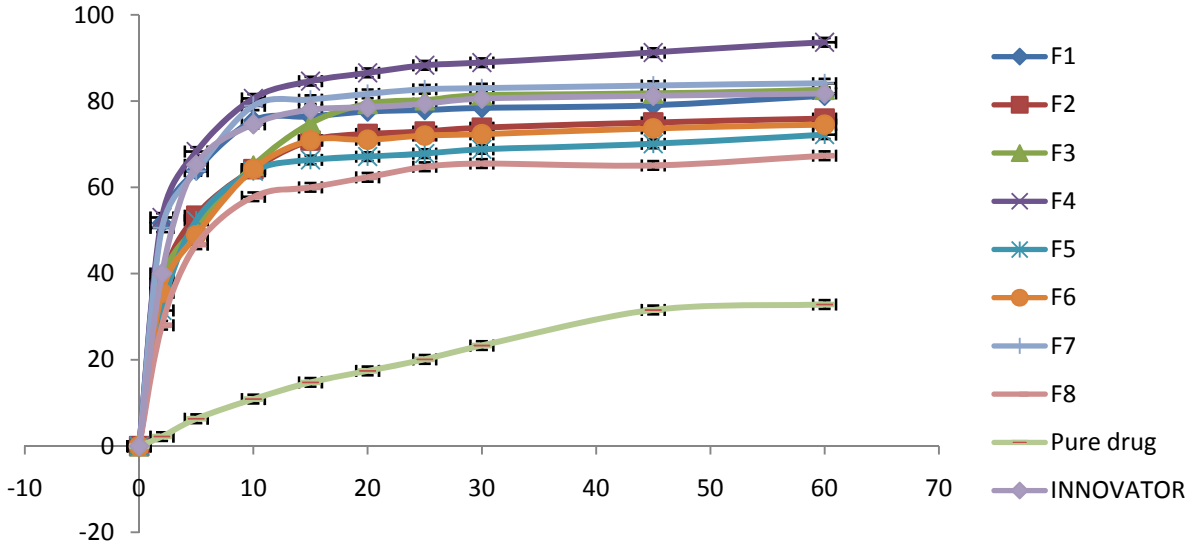


Figure 7: *in vitro* Cumulative % Drug Release for Formulations (F1-F8), pure drug and innovator

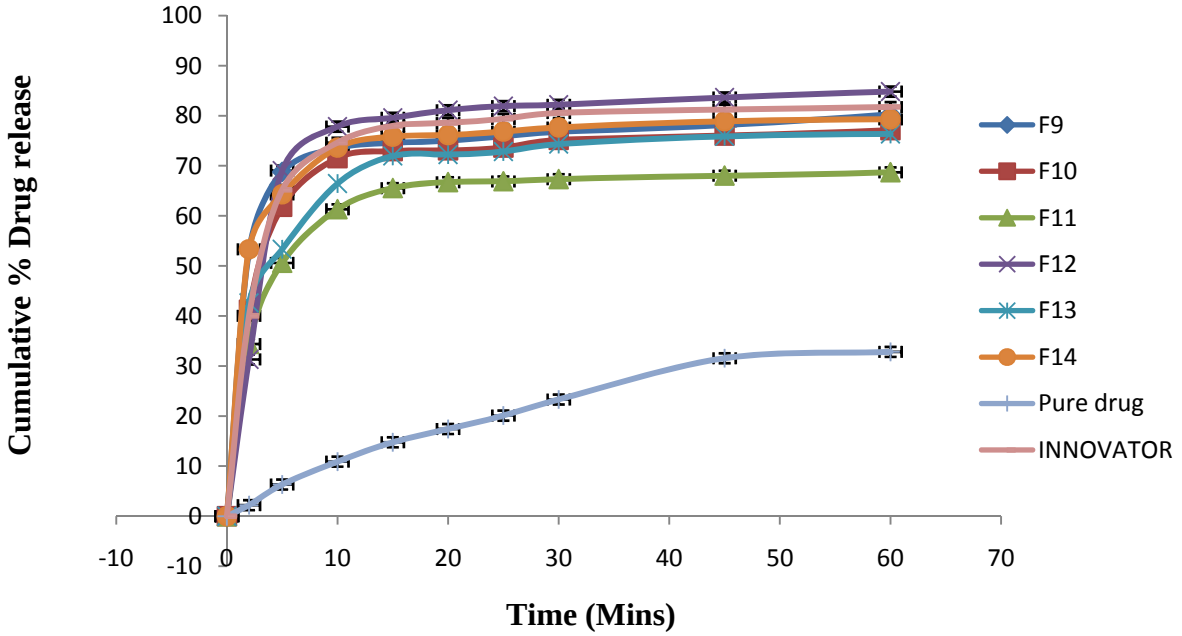


Figure 8: *in vitro* Cumulative % Drug Release for Formulations (F9-F14), pure drug and innovator

## Particle size analysis of SMEDDS

The particle size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption. The average particle size of SMEDDS for transparent micro-emulsions should be less than 50nm. The particle size of the optimized SMEDDS formulation was found to be 19.5nm indicating all the particles were in the micrometer range. Figure 9 represents the particle size analysis of optimized SMEDDS formulation.

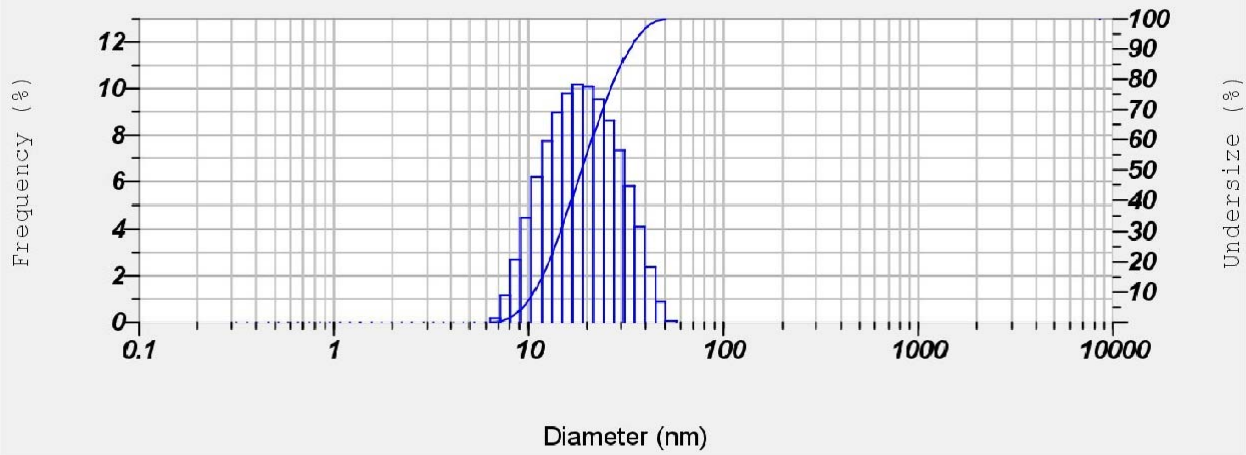


**Figure 9: Particle size analysis of optimized Nevirapine SMEDDS formulation (F4)**

## Zeta potential of SMEDDS

Zeta potential has got practical application in the stability of emulsion since it governs the degree of repulsion between adjacent, similarly charged and dispersed droplets. In general, the zeta potential value of ±30 mV is sufficient for the stability of a micro emulsion. The zeta potential of the optimized SMEDDS formulation was found to be -32.6 mV which complies with the requirement of the zeta potential for stability. Figure 10 represents the particle size analysis of optimized SMEDDS formulation.

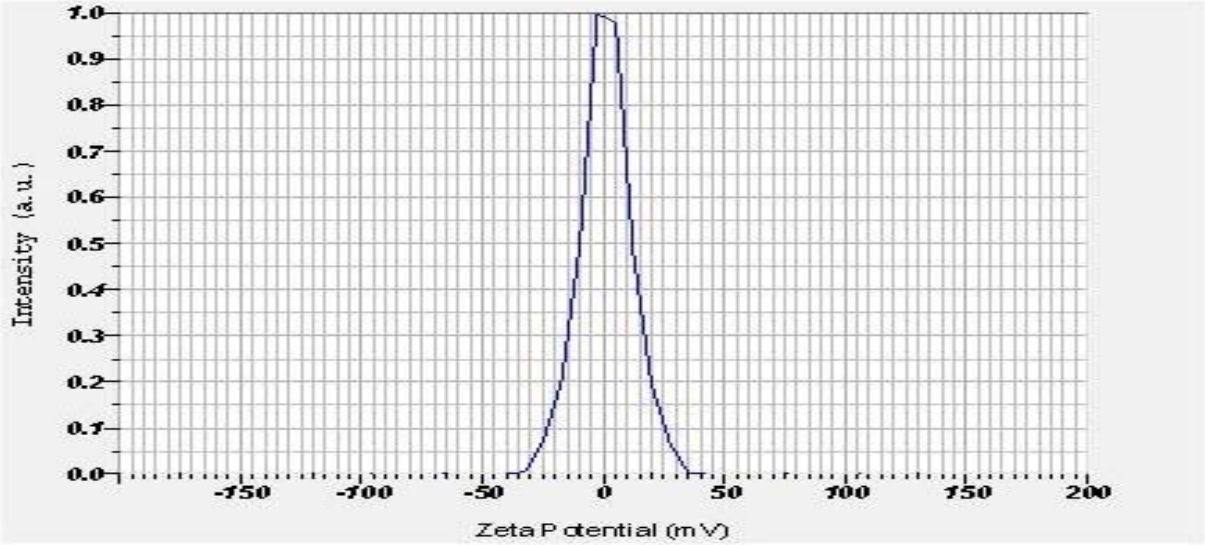


**Figure 10: Zeta potential of the optimized Nevirapine SMEDDS formulation (F4)Drug excipient interactions by FTIR spectroscopy**

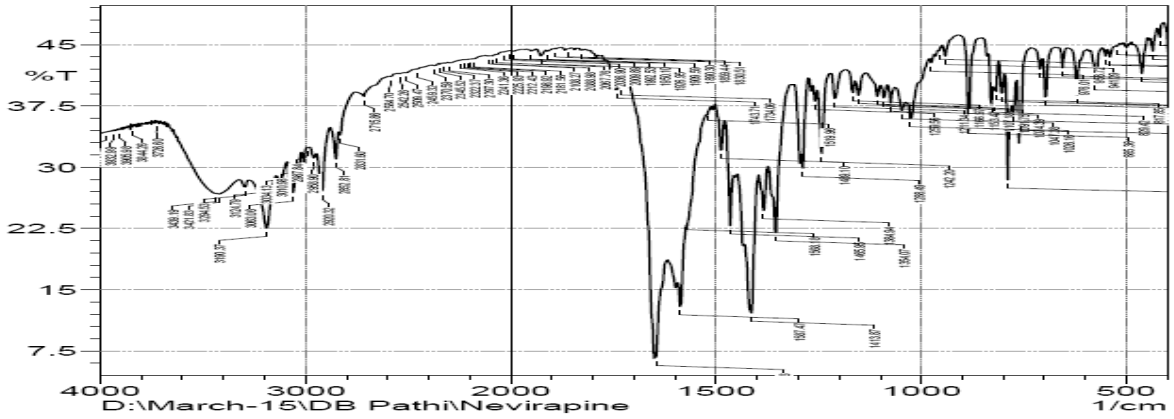


Figure 11: FTIR Spectroscopy of Nevirapine pure drug

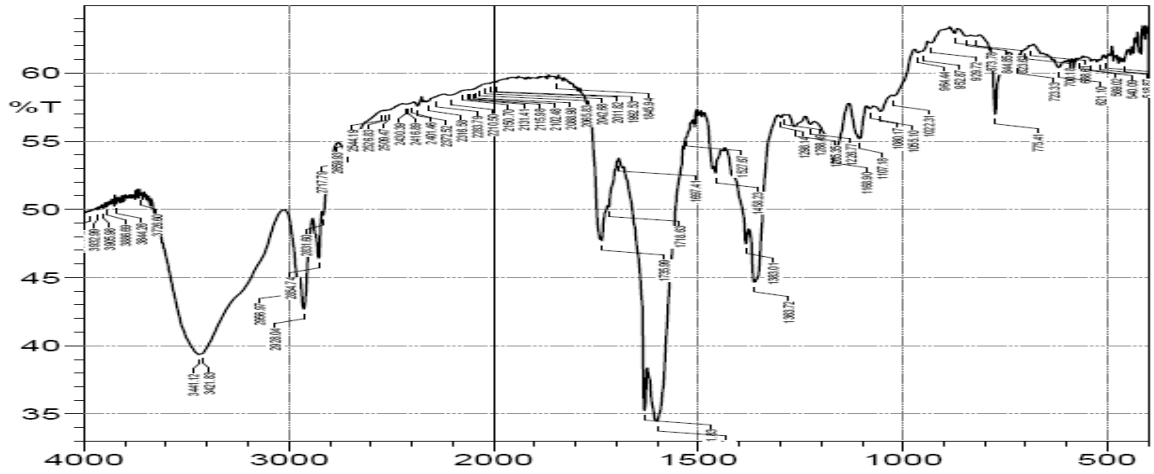


Figure 12: FTIR Spectroscopy of Nevirapine + Capryol 90 (oil)

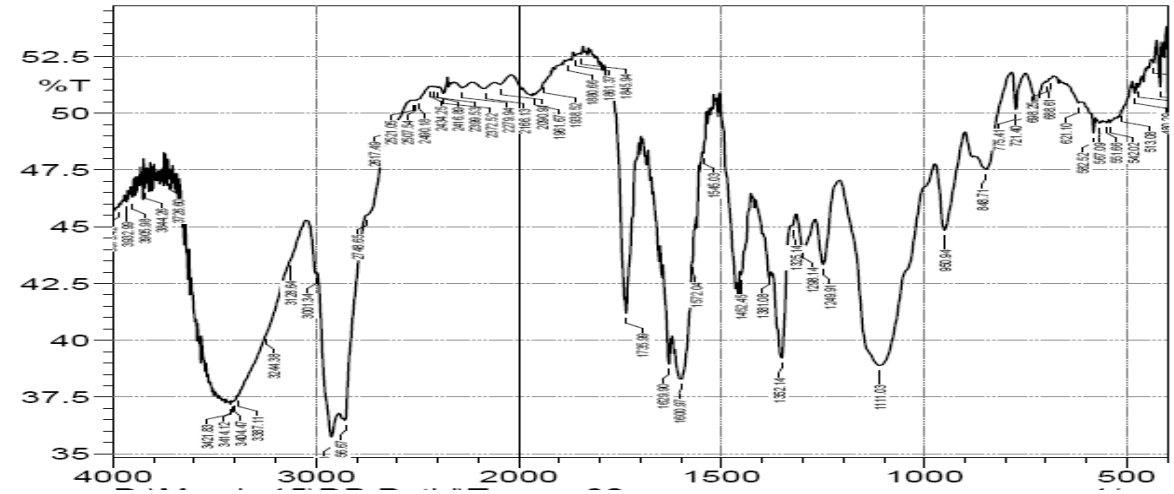


Figure 13: FTIR Spectroscopy of Nevirapine + Tween 80 (surfactant)

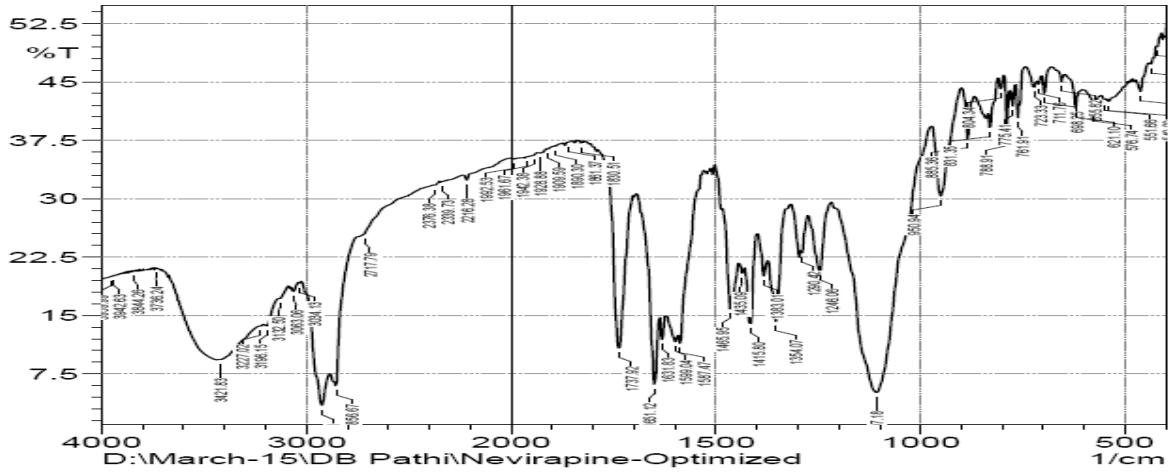


Figure 14: FTIR Spectroscopy of Nevirapine optimized formulation (F4)

## Interpretation of FTIR Data

FT-IR spectrums are mainly used to determine if there is any interaction between the drug and any of the excipient used. The FTIR spectra of pure Nevirapine (Figure 11) displayed bands at 3097cm-1 due to N-H stretch, at 1640cm-1 due to C=O stretching, at 1290cm-1 due to aromatic amine group C-N stretching. The spectra also showed bands at 1290cm-1 due to C-N bending. The FTIR spectrum of SMEDDS (Figure 14) containing Nevirapine exhibited characteristic bands consistent with the molecular structure of Nevirapine such as bands at 3095 cm-1 due to N-H stretch, at 1645cm-1 due to C=O stretching, at 1288 cm-1 due to aromatic amine group C-N stretching. FTIR spectrum of Nevirapine + Capryol 90 and Nevirapine + Tween 80 are shown in Figure 12 & 13 respectively. Thus, the presence of characteristic absorption bands of Nevirapine and the SMEDDS containing Nevirapine suggest that there was no interaction between the drug and excipients used in the formulation.

## Drug excipient interactions by DSC Studies

The DSC thermo grams of Pure Nevirapine showed in Figure 15, sharp endothermic peak at melting point (2450C), indicating that the drug is highly crystalline. The absence of drug peak in the SEDDS optimized formulation F4 indicating the drug was in amorphous form.

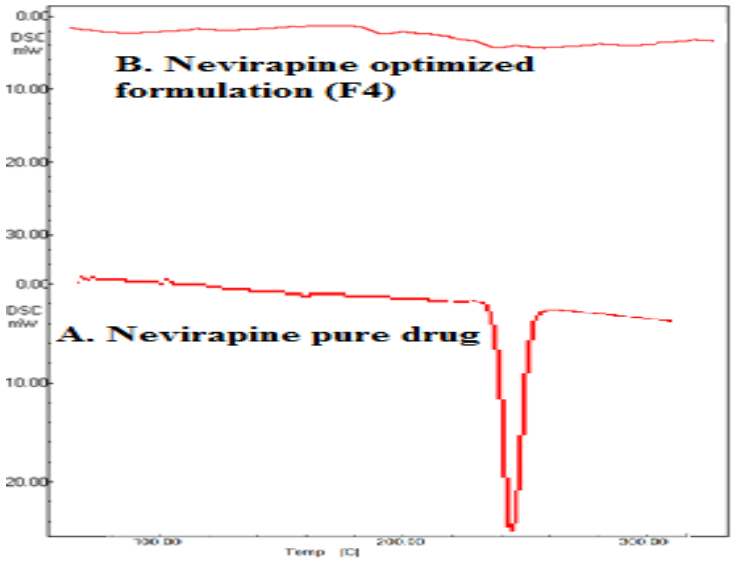


Figure 15: DSC thermo grams of Nevirapine pure drug (A) and SEDDS optimized formulation F4 (B).Scanning electron microscopy (SEM) for optimized SMEDDS (F4)

Scanning electron microscope pictures (**Figure 16**) of optimized formulation (F4) indicated that the particles are spherical and rough surface.

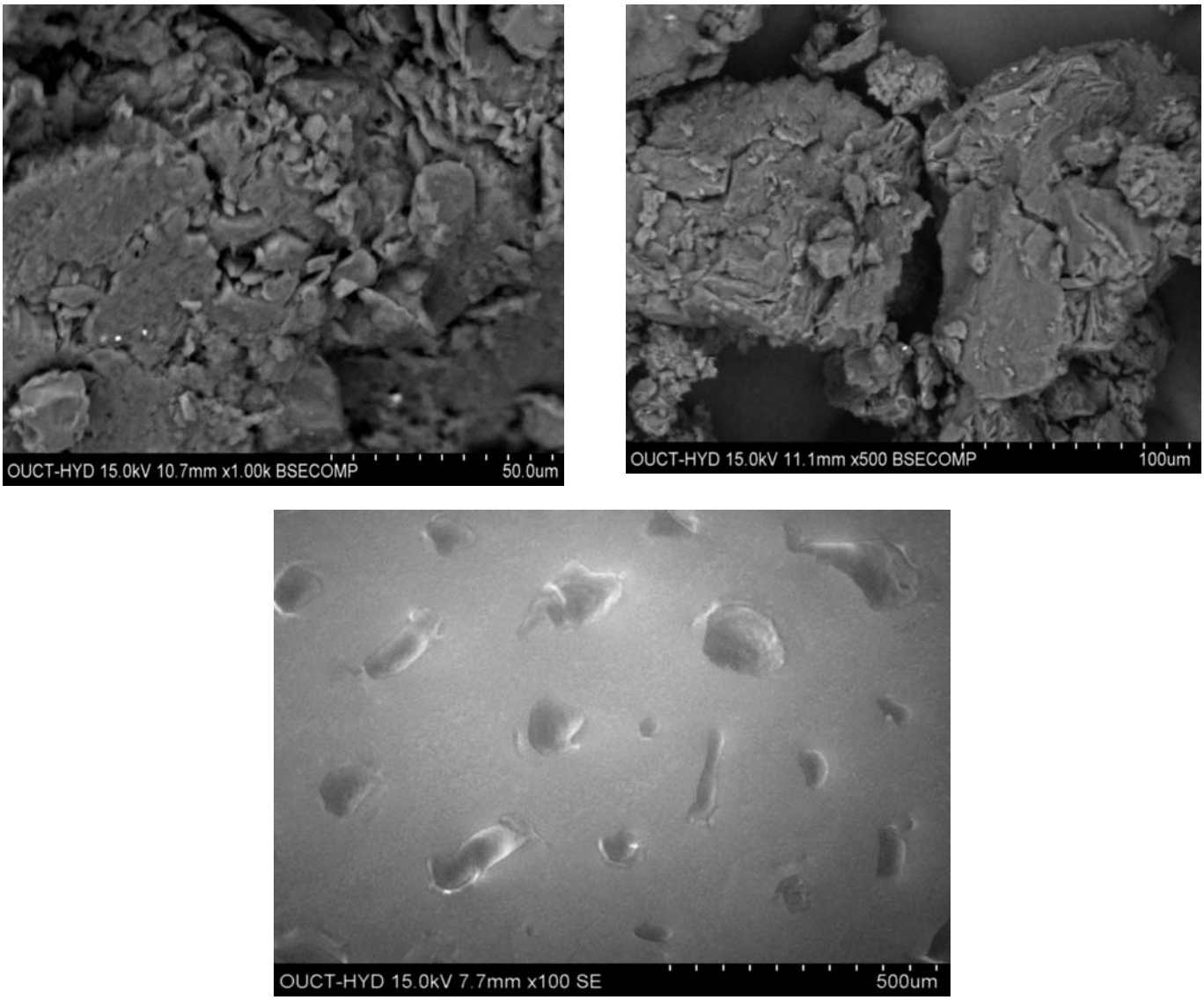


**Figure 16: Scanning Electron Microscopy of optimized SMEDDS (F4)**

## Stability studies

The optimized Nevirapine SEDDS (F4) was poured into hard gelatin capsules as the final dosage form. The developed formulation was subjected to stability studies for 6 months to evaluate its stability and the integrity of the dosage form. There was no significant change in the drug content, drug release. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. There was no significant change in the appearance or micro emulsifying property. Thus, these studies confirmed that the formulation was stable and its compatibility with hard gelatin capsules.

## *In vivo* bioavailability studies

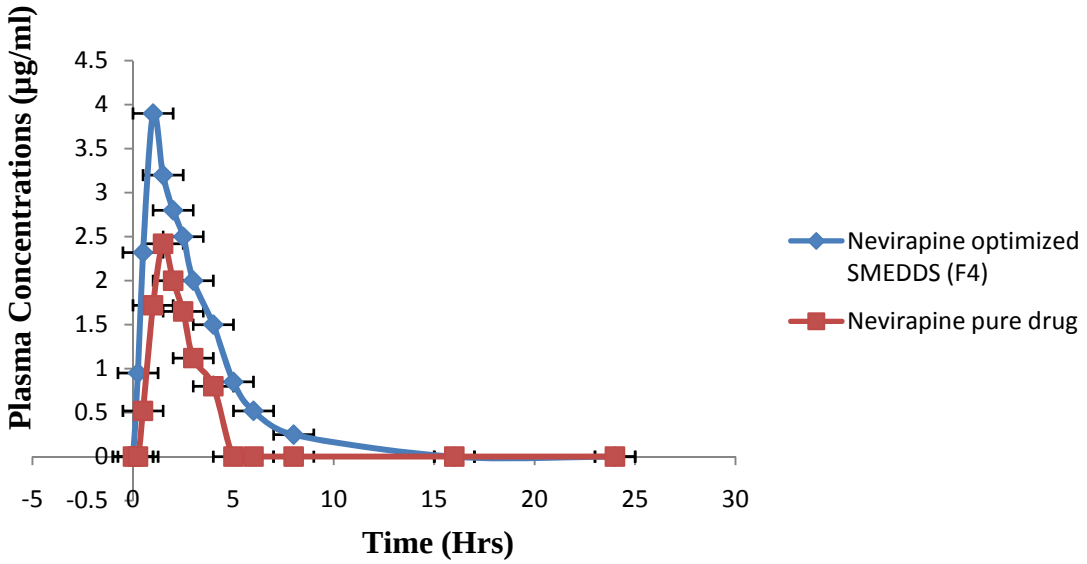


**Figure 17: Plasma Concentrations of Nevirapine optimized SMEDDS (F4) and Nevirapine pure drug at different time intervals (Mean ± SD, n = 6)**

**Table 5: Pharmacokinetic Parameters of Nevirapine optimized SMEDDS (F4) Nevirapine optimized SMEDDS (F4) and pure drug**

| Pharmacokinetic Parameters | Nevirapine optimized SMEDDS (F4) | Nevirapine Pure drug |
|---|---|---|
| $C_{max}$ (µg/ml) | 3.9±0.05 | 2.02±0.35 |
| $AUC_{0-t}$ (µg.hr/ml) | 44.7±0.06 | 22.1±0.05 |
| $AUC_{0-inf}$ (µg.hr/ml) | 45.25±0.08 | 24.25±0.07 |
| $T_{max}$ (hr) | 1.00±0.05 | 1.50±0.03 |
| $t_{1/2}$ (hr) | 2.02±0.04 | 3.15±0.01 |
| K el ($hr^{-1}$) | 0.191±0.05 | 0.364±0.02 |

## Pharmacokinetic parameters comparison for Nevirapine pure drug suspension and optimized SMEDDS (F4)

Figure 17 shows the plasma concentration–time curve in Wistar rats after a single oral dose of Nevirapine optimized SMEDDS (F4) as compared to Nevirapine pure suspension. At all the indicated time points, the Nevirapine plasma concentrations in rats treated with optimized formulation (F4) was significantly higher than those treated with pure drug. Pharmacokinetic parameters of Nevirapine after oral administration of the two formulations in Wistar rats are shown in Table 5.

As can be seen from the above table, $C_{max}$ of the optimized formulation (F4) 3.9±0.05µg/ml was significant ($p<0.05$) as compared to the pure drug suspension formulation 2.02±0.35µg/ml. $T_{max}$ of both optimized formulation (F4) and pure drug suspension was 1.00±0.05hr and 1.50±0.03hr, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration. $AUC_0$. infinity for optimized formulation (F4) was higher 45.25±0.08 µg.hr/ml than the pure drug suspension formulation 24.25±0.07µg.hr/ml. Statistically, $AUC_{0-t}$ of the optimized SMEDDS (F4) (44.7±0.06) was significantly higher ($p<0.05$) as compared to pure drug suspension formulation (22.1±0.05). Higher amount of drug concentration in blood indicated better systemic absorption of Nevirapine optimized formulation (F4) as compared to the pure drug suspension formulation. Calculated concentration was found to be more for optimized formulation (F4) compared with pure drug of Nevirapine.

## Summary and conclusion

Our studies highlighted the potential of using SEDDS as an efficient strategy for the oral delivery of hydrophobic Nevirapine. Nevirapine was formulated as SEDDS based on the oil solubility studies and ternary phase diagrams. From this study it was concluded that, prepared SEDDS was thermodynamically stable with good self emulsification efficiency and having globule size in nanometric range which may be physiologically stable. On the basis of different evaluation parameters and dissolution studies F4 was found to be optimized formulation which contains Capryol 90 as oil, Tween 80 and PEG 600 as a surfactant and co-surfactant respectively. FTIR analysis revealed that, there was no interaction

between the drug and polymers. From DSC studies it was concluded that the optimized formulation was in amorphous state, which influenced the enhancement of solubility. Results of SEM indicated that the homogeneous and spherical droplets in micro emulsion were observed. In-vitro drug release of optimized SEDDS (F4) was much higher than that of pure Nevirapine and marketed formulation. Hence it was concluded that SEDDS can be efficiently formulated to enhance dissolution rate of poorly soluble drug such as Nevirapine. The pharmacokinetic data indicated that the Nevirapine SEDDS have better in vivo absorption compared to pure drug suspension. The higher bioavailability might be due to the enhanced solubility of Nevirapine by SEDDS formulation. The oral bioavailability study of optimized SEDDS (F4) showed improvement by a factor of 2 - fold compared to the pure drug suspension in rats. Thus Nevirapine with SMEDDS may be used for improvement of oral bioavailability of drugs with poor water solubility and low oral bioavailability.

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