ADVANCE TREND IN NOVEL FORMULATION OF NEVIRAPINE CONJUGATED NANOEMUSION FOR ANTIRETROVIRAL MEDICATIONS

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Abstract

The aim of this study is to formulate and evaluate the nanoemulsion of Nevirapine by use 32 factorial design to improve the oral bioavailability of nevirapine by enhance the solubility, dissolution rate and percentage transmittance. Solubility of nevirapine in different oils, surfactant and co-surfactant was determined for the screening of excipients. As based on the solubility study castor oil as oil phase, tween20 as surfactant and PEG 400 as co-surfactant were used for the NVP formulation. Nanoemulsion of nevirapine was developed using the ultrasonication method. The efficient self-emulsification region was determined by constructing with the help of ternary phase diagram having ratio 3:1, and the particle size evaluated by the zeta sizer. In vitro release profile showed more than 98 % release within 5 h which was highly significant. The particle size of F2 formulation within range found to be 131.1nm as compared to F1, F3, F4, F5, F6, F7, F8, F9, the results suggest that F2 formulation is better with regards by comparing all the evaluation parameter.

Keywords: Nanoemulsion, Nevirapine, Spectrophotometer, Solubilization, Zeta Potential.

INTRODUCTION

NVP stands for non-nucleotide reverse transcriptase inhibitor and is used to treat HIV infection. NVP is a BCS class 2 drug, indicating it has a low solubility and a high permeability.1 As NVP is a BCS class II drug with poor water solubility and high permeability, achieving acceptable dissolution kinetics from the designed dosage form is complicated.2

The molecular formula of Nevirapine is C15H14N4O and Molecular weight is 266.304 g·mol−1.3 with pKa Value 5.06.4 Nevirapine is soluble in organic solvents such as DMSO and dimethyl formamide (DMF) and partially soluble in methanol.5

The human immunodeficiency virus (HIV), a retrovirus of the lenitivirus family, causes particular damage to the immune system, culminating in acquired immunodeficiency syndrome (AIDS). HIV is a retrovirus that primarily infects CD4+ T cells, macrophages, and dendritic cells, all of which are important components of the human immune system. It kills CD4+ T cells both directly and indirectly. CD4+ T cells are essential for the immune system to operate properly. Human immunodeficiency virus type 1 (HIV-1) infects 40 million individuals worldwide.6

Around 40% of novel chemical entities have low water solubility, posing a significant barrier to contemporary drug delivery systems. The solubilization of these medications in the (GI) tract is frequently the rate limiting step in their absorption. Lipid-based drug delivery systems have been shown to improve the bioavailability of highly lipophilic substances by allowing the medication to remain dispersed until it is absorbed, thereby overcome the barrier of slow dissolution rates. Lipid formulations can range from pure oils to formulations comprising surfactants, co-surfactants, or co-solvents in various amounts. A number of recent lipid formulation research have concentrated on microemulsion formulations, with a focus on self-emulsifying or self-emulsifying drug delivery systems (SEDDS) to increase oral bioavailability of poorly water soluble medicines.7

A miniemulsion, or fine oil/water or water/oil dispersion stabilised by an interfacial coating, is also known as a nanoemulsion. Droplet size range of 20–600 nm of surfactant molecule. NEs are transparent due to their tiny size. 8 There are three types of nanoemulsions that can be created:9

a) Oil in water NE

b) Water in oil NE

c) Bi- continuous NE

MATERIALS AND METHODS:

Nevirapine was obtained from sigma-aldrich. Castor oil was obtained from Molychem, Tween 20 was obtained from PALLAV Chemicals, Polyethylene glycol (PEG) 400 was obtained from THOMAS BAKER Chemicals.

Methods:

Melting point

Melting point of the NVP were estimated using the digital melting point apparatus. The observed value of nevirapine was 247º C.

Solubility study

Solubility of drug is an important parameter for the preparation of nanoemulsion. Solubility of poorly soluble drugs into the oil, surfactant and co-surfactant. Add 1mg of drugs into 1ml of each oil, surfactant and co-surfactant and mixed well. Then applied heat with the help of water bath and vigorous shaking and Cooled the sample at room temperature. After that pipetted out 0.1ml of prepared sample into the 10 ml volumetric flask and made volume with 0.1N HCL, Prepared solution kept on the magnetic stirrer for proper mixing (2-5min). Filtered the sample with help of whatman filter paper. The absorbance of NVP determined by UV Spectrophotometer at 282 nm.10

Construction of pseudo-ternary phase diagrams

In order to find out the concentration range of components for the existing range of nanoemulsions, pseudo-ternary phase diagram was constructed using the water titration method. Ternary plots were constructed using oil, surfactant and co-surfactant containing different proportion of surfactant: Co-surfactant, that is, Smix (1:1, 2:1, 3:1 w/w). In brief Smix and oil were mixed at ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 in preweighed test tube. The mixtures of oil and Smix at certain weight ratios were diluted with water, under moderate stirring. After being equilibrated, the mixtures were assessed visually and determined as being nanoemulsions or coarse emulsions. The data obtained was used for the construction of ternary plots with the help of Chemix School software.11

Preparation of stock solution

An accurately weighted of 100mg of nevirapine was taken into the 100ml of volumetric flask and dissolved with small proportion of methanol and makeup volume with water. An above solution 10ml pipetted out into the 100 ml volumetric flask and makeup volume with water. After that Pipette out 0.1, 0.2, 0.3, 0.4, 0.5 ml of solution from working standard into the 10 ml of volumetric flask and makeup volume with water to obtain 1, 2, 3, 4 or 5 ppm solution respectively. Then standard solution of nevirapine was scanned in the UV range 200-400nm and overlain the spectrum with 282nm as a λ max.

IR Spectroscopy

The purpose of the FT-IR experiments was to characterize the drug. FT-IR spectroscopy was employed to determine the functional group and bonds between the elements. The sample was scanned by using opus software. IR spectrophotometer between 4000-400 cm-1 with resolution of 4 cm-1.12

Preparation method of nanoemulsion

NE was formulated using oil phase (castor oil 3%), surfactant (tween 20), co-surfactant (PEG 400) and water. The nanoemulsion was formulated by the adding water in the mixture of oil and Smix in different proportions, 1:1, 2:1, and 3:1(v/v) respectively. Mixed it well, and mixture was sonicated using a 20 kHz sonicator (probe sonicator)11 with maximum power output of 750 W for 30 min at room temperature. During the sonication process heat energy generated was neutralized by keeping the sample container in an ice bath. The formulated nanoemulsion droplet size and stability were analysed, and nanoemulsion was used further for in vitro studies.

Percentage transmittance

Percentage transmittances (% T) of NE shows the transparency of the solution. The sample were diluted 10 times with distilled water for percentage transmittance (% T) measurement, and % T was confirmed against distilled water using a UV spectrophotometer at 282 nm.12

Viscosity

The viscosity is determined of different formulations by Brookfield DV III ultra V6.0 RV CONE at 25±1 at 10 rpm for 5 min.

Data mentioned in table 4.13

In vitro dissolution study

In vitro dissolution study of NVP nanoemulsion was conducted by the dialysis technique. 0.1N HCL was used as dialysis medium. The selected sample was placed in dialysis tubing (Dialysis membrane 12,000-14,000 daltons; pore size: 2.4 nm) which was closed by the clamped. Another end of tube was also secured with dialysis closure clips and placed in 900 ml of dialyzing media, which was agitated at 50 rpm using a magnetic stirrer at 37º C. At 30 minute time intervals, 5 mL aliquots were removed and diluted further. The dialyzing media was replenished every time the volume of aliquots was reduced. A UV-visible spectrophotometer (JASCO 630) set to 282 nm. It was used to examine the sample of NVP in the dialyzing liquid at the appropriate time.

RESULTS AND DISCUSSION

Screened oil, surfactant and co-surfactant

The result of the solubility of nevirapine in appropriate vehicles were showed in below table. From the solubility data; Castor oil, tween 20, and PEG 400 were selected as oil phase, surfactant, and co-surfactant respectively. As per obtained results solubility of nevirapine in castor oil (6.9625), tween 20 (15.4626) and PEG 400 (7.4773) is greater than another oil, surfactant and co-sufactant. (Table 1, 2, 3)

Table 1. Solubility of NVP in oils

Solubility of nevirapine in different oils	
Oil	**Solubility in mg/ml ($\bar{X}$ ± SD)**
Soyabean oil	1.1046±0.061
Castor oil	6.9625±0.032
Arachis oil	1.14628±0.042
Olive oil	0.9022±0.005
Rose oil	5.3419±0.072
Linseed oil	5.6271±0.028

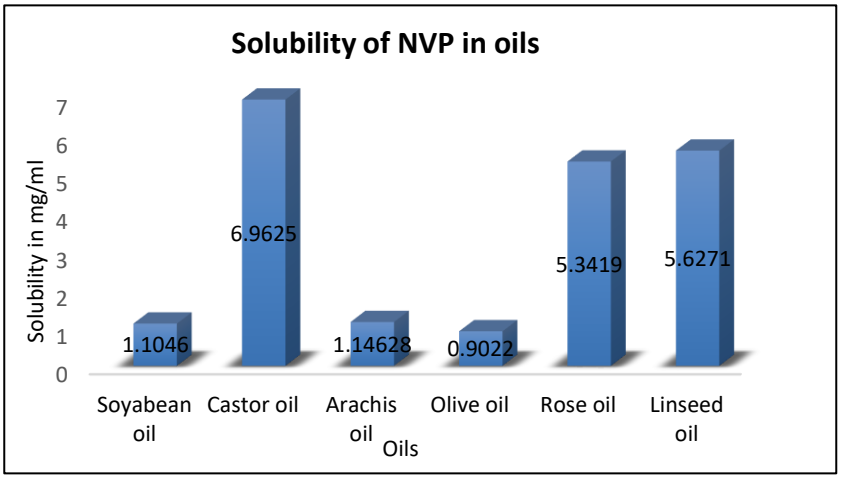

Figure 1: Solubility graph of NVP in oils

Table 2: Solubility of NVP in surfactants

Solubility of Nevirapine in different surfactants	
Surfactant	**Solubility in mg/ml ($\bar{X}$ ± SD)**
Tween 20	15.4626±0.03
Tween 60	9.0578±0.051
Tween 80	9.6959±0.046
Span 20	13.6683±0.07
Span 80	13.4714±0.061

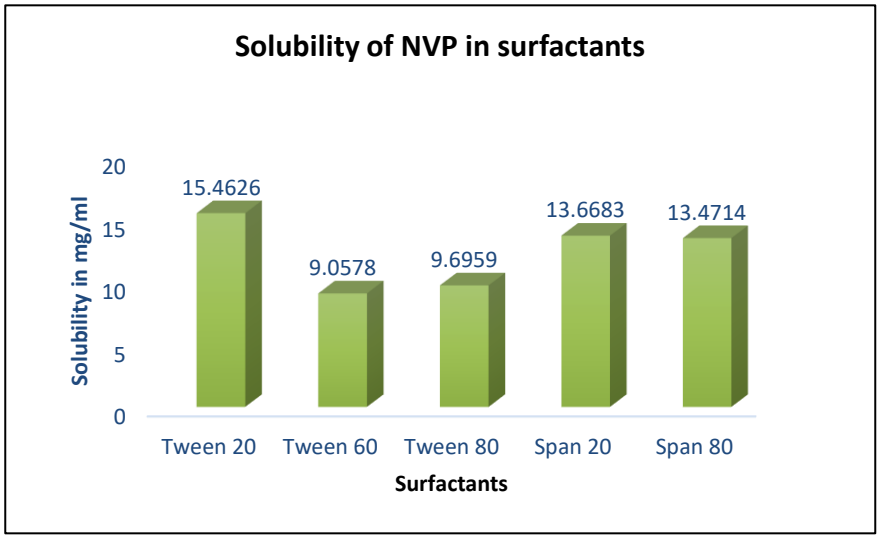

Figure 2: Solubility graph of NVP in surfactants

Table 3. Solubility of NVP in co-surfactants

Solubility of nevirapine in different co-surfactants	
Co-Surfactant	**Solubility in mg/ml ($\bar{X}$ ± SD)**
PEG 200	6.9899±0.94
PEG 400	7.4773±0.89
PEG 600	7.0689±1.27

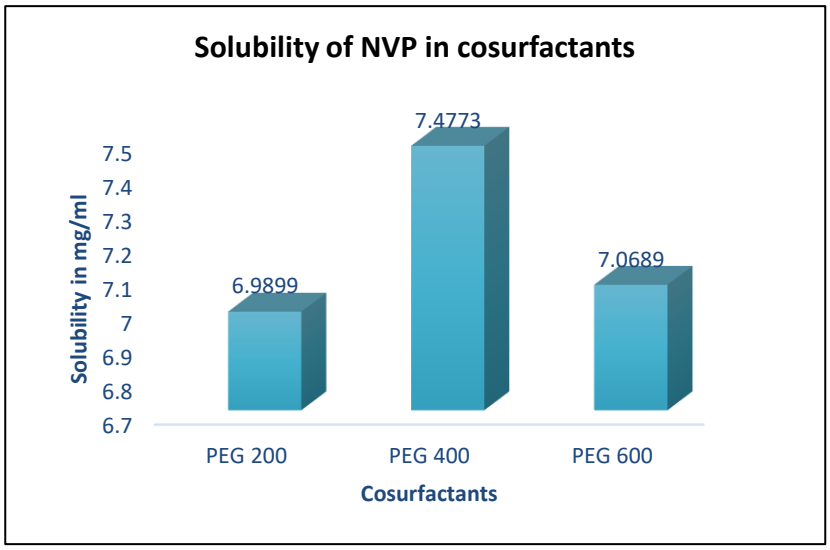

Figure 3. Solubility graph of NVP in co-surfactants

Ternary plots

Figures 4. illustrate phase diagrams of systems that contain Castor oil as an oil phase, Tween 20 as a surfactant, and PEG 400 as a co-surfactant at surfactant/co-surfactant Smix ratios of 1:1, 2:1 and 3:1 (w/w) to determine the presence of a nanoemulsion region. When compared to all other ternary plots, the obtained nanoemulsion region at Smix ratios of 1:1 were low, according to the phase research. The rise in the nanoemulsion regions as the concentration of cosurfactant increases at Smix ratios of 2:1, and 3:1 [Figure 3] indicating that the cosurfactant has some effect on the ability to develop nanoemulsion. When compared to all other ternary plots, the ratio 3:1 of Smix revealed the largest nanoemulsion region. An increased the surfactant concentration leads to the larger nanoemulsion regions. When compared to all other ratios, the 3:1 Smix ratio demonstrated the most nanoemulsion region in the experiments. Based on nanoemulsion region formation capabilities, a Smix ratio of 3:1 was chosen

for the NVP formulation, and it was taken to additional research.

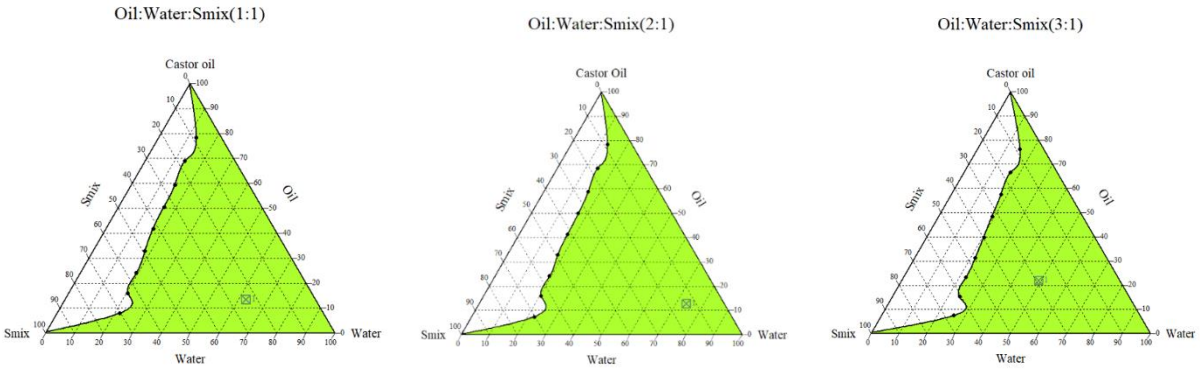

Figure 4. Ternary plots of Tween 20: PEG 400 in different ratios (1:1, 2:1, 3:1 respectively)

Percentage transmittance

The percentage transmittance of the optimized nevirapine nanoemulsion was found to be 96.69±1.96 %. The value of % transmittance closer to 100% shows that the optimized nanoemulsion was clear and transparent. (Table 6)

Cloud point measurement

The cloud point is the temperature above the aqueous solution and formulation becomes cloudy. The optimized NVP formulations were compared for the cloud point value. All the formulations were diluted with water in the ratio of 1:100 and placed in the water bath with gradually increasing in the temperature. (Table 6)

In vitro dissolution data of nevirapine nanoemulsion

In vitro dissolution study was performed to compare the drug release from the developed NVP nanoemulsion. In vitro dissolution study of the NVP nanoemulsion (F1, F2, F3, F4, F5, F6, F7, F8 and F9) was performed by using a dialysis technique. The drug release results of In vitro disssolution study were listed in Table 7 and figure 8. After analysing the data, it was discovered that the NVP F2 better formulation compared to all other formulation, it released nearly 56.64±0.48 percent of the drug within 1 hour, compared to the other formulations.(figure 5)

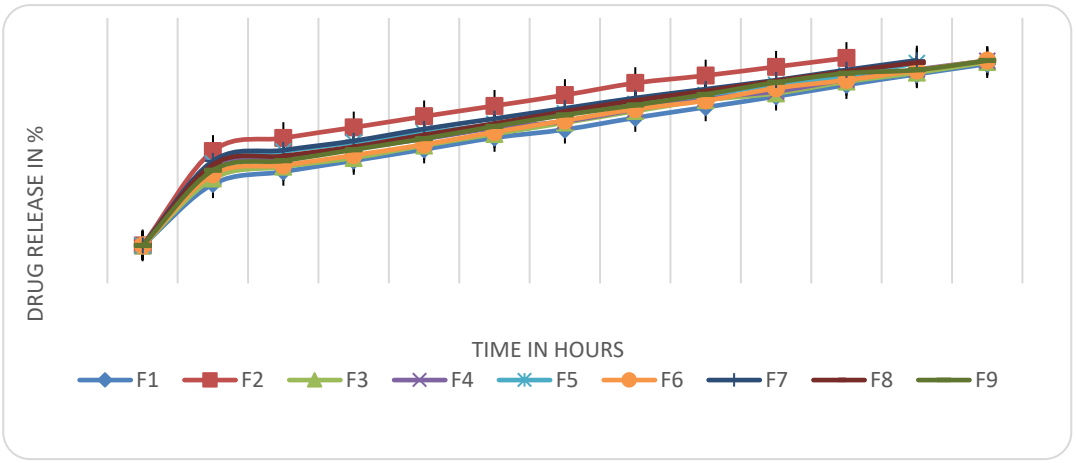

Figure 5: comparative in vitro dissolution profile plot of different formulations of Nevirapine in 0.1N HCL

Fourier transform infrared studies (FT-IR)

The purpose of the FT-IR experiments was to characterize the drug. Here, we're examining at FT-IR investigations of the pure drug is C-H bending (787.61 cm-1) 1, 2, 3 tri-substituted, C=N Stretching (1644.29 cm-1) amine /oxime, N-H bending (1585.29 cm-1) and C=O stretching (1644.29 cm-1). (Figure 6)

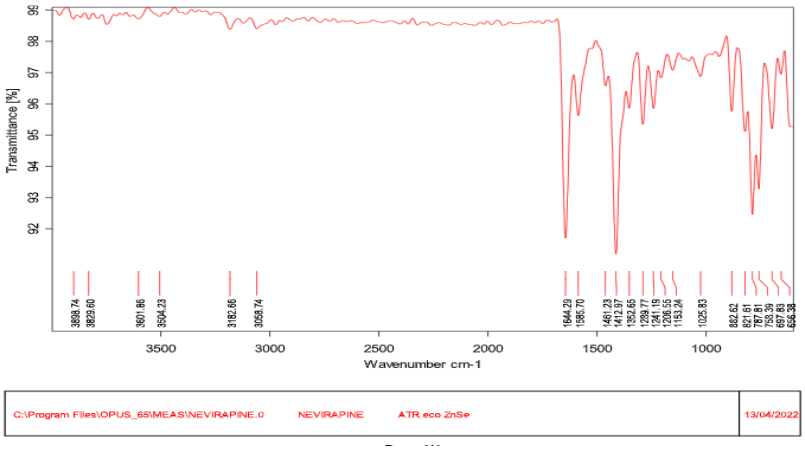

Figure 6: Fourier infrared graph of Nevirapine pure drug

Droplet size and zeta potential determination:

Zeta potential measurements were used to determine the charge of the droplets. Droplet size and the zeta potential of the formulated nanoemulsion were determined by the use of HORIBA SZ-100 for window (Z Type) Ver2.40. Light scattering was measured at a 90° angle at 25°C. The results of SEDDS formulations were showed in Table no 1. The F2 formulation was found to be the best when compared to all other formulations, with a droplet size of optimized nanoemulsion was found to be 131.1nm and a zeta potential of 24.2 mV, according to the data collected through (Figures 7)

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	127.8 nm	29.5 nm	125.5 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	127.8 nm	29.5 nm	125.5 nm

Cumulant Operations

Z-Average : 131.1 nm

PI : 0.229

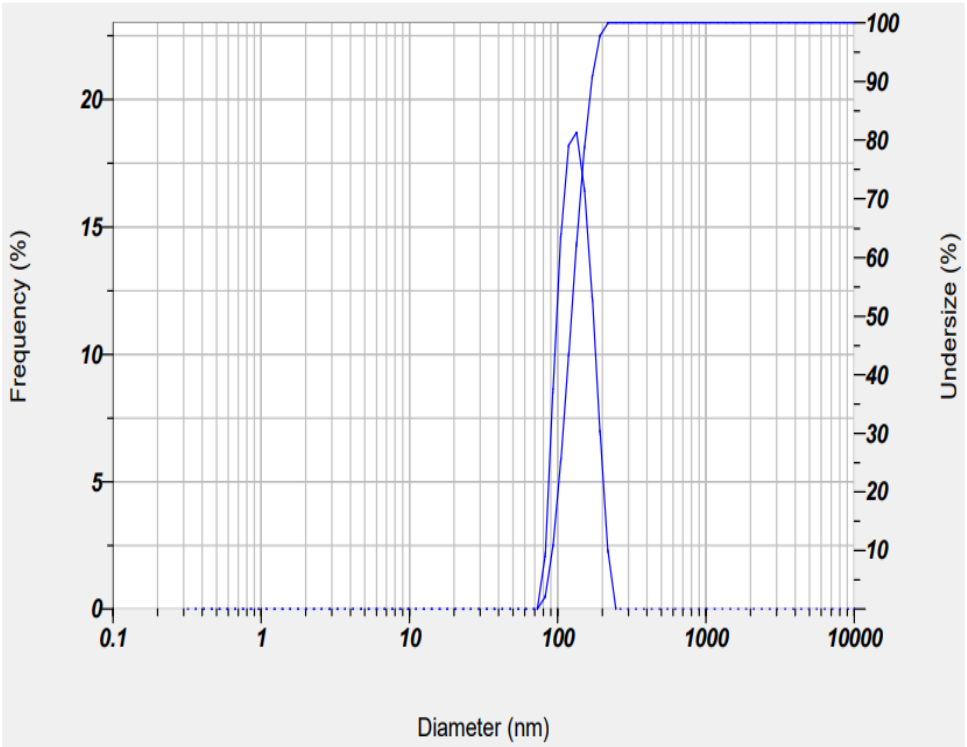

Figure 7: Particle size

Table 4: Evaluation parameters of NVP nanoemulsion

Formulation	Particle size (nm)	% Transmittance (%T)	Cloud point	Viscosity (cps)
F1	133.3	94.78±1.58	89°C	26.44±0.27
F2	131.1	96.69±1.96	92°C	33.20±0.31
F3	132.9	93.25±1.69	88°C	28.32±0.14
F4	135.6	91.89±1.14	90°C	32.71±0.21
F5	134.2	92.67±1.36	85°C	29.56±0.64
F6	137.7	90.77±1.23	87°C	31.19±0.46
F7	134.9	92.12±1.45	82°C	29.99±0.48
F8	133.8	94.88±1.64	89°C	30.46±0.34
F9	138.2	90.24±1.18	86°C	27.06±0.32

CONCLUSION

In this article we have conclude that the formulation of nevirapine nanoemulsion. The nanoemulsion shows the good oral bioavailability. The preparation is taken under consideration by screening of oil, surfactant and co-surfactant and evaluating the various parameter such as, particle size, percentage transmittance, cloud point, viscosity, pseudo-ternary phase diagrams, and dissolution studies. The evaluation parameter value is in limit. Prior to this values, result suggest F2 show better formulation comparing to others.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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