Original Research Article

Formulation, optimization, and evaluation of self-emulsifying drug delivery systems of nevirapine

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Abstract

Background: The aim of the present study was to formulate and optimize the self-emulsifying drug delivery systems (SEDDS) of nevirapine (NVP) by use of 2^2 factorial designs to enhance the oral absorption of NVP by improving its solubility, dissolution rate, and diffusion profile. SEDDS are the isotropic mixtures of oil, surfactant, co-surfactant and drug that form oil in water microemulsion when introduced into the aqueous phase under gentle agitation. **Materials and Methods:** Solubility of NVP in different oils, surfactants, and co-surfactants was determined for the screening of excipients. Pseudo-ternary phase diagrams were constructed by the aqueous titration method, and formulations were developed based on the optimum excipient combinations with the help of data obtained through the maximum micro emulsion region containing combinations of oil, surfactant, and co-surfactant. The formulations of SEDDS were optimized by 2^2 factorial designs. **Results:** The optimum formulation of SEDDS contains 32.5% oleic acid, 44.16% tween 20, and 11.9% polyethylene glycol 600 as oil, surfactant, and co-surfactant respectively. The SEDDS was evaluated for the following drug content, self-emulsification time, rheological properties, zeta potential, *in vitro* diffusion studies, thermodynamic stability studies, and *in vitro* dissolution studies. An increase in dissolution was achieved by SEDDS compared to pure form of NVP. **Conclusion:** Overall, this study suggests that the dissolution and oral bioavailability of NVP could be improved by SEDDS technology.

Key words: 2^2 factorial designs, nevirapine, oleic acid, polyethylene glycol 600, self-emulsifying drug delivery systems, tween 20

INTRODUCTION

Nevirapine (NVP) is a nonnucleotide reverse transcriptase inhibitor for the treatment of HIV infection. NVP is a biopharmaceutical classification system (BCS) class 2 drug, that is, low solubility and high permeability.[1-3] Oral route is the most oldest and convenient route for the administration of therapeutic agents due to low cost of therapy and ease of administration leads to a higher level of patient compliance.[4]

Approximately, 40% of new chemical entities exhibit poor aqueous solubility and present a major challenge to modern drug delivery system. The rate limiting step for the absorption of these drugs is often their solubilization in the gastrointestinal (GI) tract. These drugs are classified as class II drug by BCS, drugs with poor aqueous solubility and high permeability. Lipid-based drug delivery Systems have been demonstrated to be useful in enhancing the bioavailability of highly lipophilic compounds because they can keep the drug in the dissolved state until it is absorbed, thus overcoming the barrier of slow dissolution rates. In practice, lipid formulations range from pure oils to formulations containing some proportions of surfactants, co-surfactants or co-solvents. Recently, a number of studies related to lipid formulations focused attention on microemulsion formulations with particular emphasis on self-emulsifying or self-emulsifying drug delivery systems (SEDDS) to improve oral bioavailability of poorly water soluble drugs. [5]

Therefore, it is necessary to develop alternative oral routes of administration to enhance the bioavailability of poorly water-soluble drugs, and furthermore obtain more successful therapeutic effects. The use of SEDDS is one of the most interesting approaches to improving the solubility, dissolution, and oral absorption for poorly water-soluble drugs.[6-8]

Self-emulsifying drug delivery systems are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants or, alternatively, one or more hydrophilic solvents and co-solvents/ surfactants. On mild agitation followed by dilution in aqueous

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media such as GI fluids, these systems can form fine oil-in-water (o/w) emulsions or microemulsion.[9] It is thought that the microemulsion is spontaneously formed by the combined action of the specific pharmaceutical excipients with low free energy.[10] The microemulsion droplets dispersed in the GI tract provide large surface area and promote a rapid release of dissolved form of the drug substance and/or mixed micelles containing drug substance, and they may be also responsible for transporting the drug through the unstirred water layer to the GI membrane for absorption. In addition to the enhanced dissolution of drugs by SEDDS, another factor contributing to the increasing bioavailability is that the lymphatic transport is responsible for a portion of the entire drug uptake as well. The lipid composition of SEDDS may be related to facilitate the extent of lymphatic drug transport by stimulating lipoprotein formation and intestinal lymphatic liquid flux.[11,12]

The main objective of the present study is to develop and evaluate an optimal SEDDS formulation containing NVP.

MATERIALS AND METHODS

Materials

Nevirapine was obtained from Cipla Ltd., Pune. Oleic acid was obtained from LOBA Chemie Pvt. Ltd., Mumbai. Tween 20 was obtained from S D Fine Chemicals Ltd., Mumbai. Polyethylene glycol (PEG) 600 was obtained from S D Fine Chemicals Ltd., Mumbai.

Methods

Solubility studies

The most important criterion for the screening of components for micro emulsion is the solubility of poorly soluble drug in oils, surfactants, and co-surfactants. The solubility of NVP in various oils was determined by adding an excess amount of drug in 2 ml of selected oils and surfactants and co-surfactants in 5 ml capacity stopper vials, and mixed using a vortex mixer. The vials containing samples were then kept at 25°C ± 10°C in an ultra-sonicator for 48 h to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 5000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 μm membrane filter. The concentration of NVP in the samples was determined using ultraviolet (UV) spectrophotometer by measuring the absorbance of samples at 313 nm.[13]

Construction of pseudo-ternary phase diagrams

In order to find out the concentration range of components for the existing range of microemulsions, 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, S/Co (1:4, 1:3, 1:2 1:1 and 4:1 w/w). In brief S_{mix} 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 surfactant and co-surfactant at certain weight ratios were diluted with water, under moderate stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions or coarse emulsions. The data obtained was used for the construction of ternary plots with the help of Triplot V4.1 software (Todd Thompson).

Formulation and optimization of nevirapine self-emulsifying drug delivery system by using 2^2 full factorial designs

It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using a minimum number of man-hours and raw materials. Traditionally pharmaceutical formulations after developed by changing one variable at a time approach. The method is time-consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to develop an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is, therefore, very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of a number of variables and their interactions.

Self-emulsifying drug delivery systems formulations were developed based on the microemulsion regions and maximum amount of drug that can be solubilized in a particular ratio of surfactant and co-surfactant with oil meeting the desired criteria for formation of microemulsion after dispersing in aqueous media. The developed formulation consisted of NVP and the selected excipients obtained through screening of solubility studies and by plotting pseudo ternary phase diagrams. Optimum ratios of oil and S/CoS were selected from the phase diagrams. SEDDS formulations were prepared by dissolving NVP in S/CoS mixtures along with gentle vortexing and heating at ≤90°C, and then by adding Oil. To study the effects of the formulation variables, different batches were prepared using 2^2 factorial designs, with each batch containing NVP and varying amounts of oil and S/CoS. Formulations were stored in a desiccator at ambient conditions for further study.

The formulation was prepared by dissolving the NVP in the mixture of Surfactant and Co-surfactant at 50°C in a water bath. Oil was then added. This mixture was mixed by cyclomixer until a transparent preparation obtained. The prepared NVP SEDDS were loaded in hard gelatin capsule.

Characterization of self-emulsifying drug delivery systems

Drug content

Self-emulsifying drug delivery systems formulation equivalent to 100 mg of NVP was taken and dissolved in small quantity of methanol. Volume was made up to 100 ml with 0.1N HCl (1 mg/ml). From the above stock solution, 0.2 ml (200 μg/ml) was withdrawn and diluted up to 10 ml with methanol (20 μg/ml). Samples were prepared in triplicate and absorbance measured

at 313 nm using UV-visible spectrophotometer. 0.1N HCl was used as a reference solution. [14]

Drug excipient compatibility studies

A proper design and formulation of the dosage form requires considerations of the physical, chemical and biological characteristics of both drug and excipients used in the fabrication of the product. Compatibility must be established between the active ingredient and other excipients to produce a stable, efficacious, attractive and safe product. If the excipients(s,) are new and if no previous literature regarding the use of those particular excipients with an active ingredient is available, then compatibility studies are of paramount importance. Infrared (IR) is related to covalent bonds, the spectra provided detailed information about molecular structure. Hence, before producing the actual formulation, compatibility of NVP with different polymers and other excipients were tested using the Fourier transform infrared (FT-IR) spectroscopy technique.

Fourier transforms infrared spectroscopy is a useful analytical technique utilized to check the chemical interaction between drug and other excipients used in the formulations. Drug and the intended excipients interaction were studied by FT-IR. The intended samples were powdered and intimately mixed with dry powdered potassium bromide. The powdered mixture was taken in a diffuse reflectance sampler, and the spectrum was recorded by scanning in the wavelength region of 4000-400 cm^{-1} in FT-IR spectrophotometer.

Determination of droplet size and zeta potential

The charge of the droplets was determined by zeta potential measurement. Zeta potential helps in predicting the flocculation effect and stability in emulsion systems. Colloid will aggregate due to attractive forces if the zeta potential falls below a certain level. Droplet size and the zeta potential of the formed emulsion were determined using a Zetasizer ZS 90 (Malvern Instruments, UK). Light scattering was monitored at 25°C at a 90° C angle.[15]

In vitro diffusion study

In vitro diffusion study of the NVP SEDDS was performed by use of dialysis technique. 0.1N HCl was used as a dialysis medium. One end of dialysis tubing (Dialysis membrane 70, HIMEDIA; MWCO 12,000-14,000 daltons; pore size: 2.4 nm) was clamped and then the experimental formulation sample, was placed in it. The other end of the tubing was also secured with dialysis closure clips (HIMEDIA, Mumbai) and was placed in 900 ml of dialyzing medium and stirred at 50 rpm over a magnetic stirrer (Remi Instrument Ltd., Mumbai, India) at 37°C. Aliquots of 5 ml were removed at 30 min time intervals and suitably diluted further. Each time the volume of aliquots was replaced with the fresh dialyzing medium.[16] These samples were analyzed for NVP present in the dialyzing medium at corresponding time by UV-visible spectrophotometer at 313 nm.

Determination of self-emulsification time

The emulsification time of SEDDS was determined according to USP XXIII, dissolution apparatus type II. Each formulation added drop-wise to 900 ml of 0.1N HCl at 37°C. Gentle agitation was provided by a standard stainless steel dissolution paddle at 50 rpm. Emulsification time was assessed visually.[17]

Rheological properties determination

The SEDDS systems were loaded in hard gelatin capsules in the present study. So, it can be easily pourable into capsules, and such systems should not be too thick. Viscosity studies are necessary for SEDDS to characterize the system physically and to control its stability. The rheological properties (viscosity, flow) of the microemulsion are evaluated by use of Brookfield viscometer (Japan) DV-E use of spindle RV-6 at 100 rpm at 25°C ± 0.5°C. This viscosities determination conform whether the system is w/o or o/w. If the system has low viscosity then, it is o/w type of the system and if a high viscosity then it is w/o type of the system.[18]

Thermodynamic stability studies

The physical stability of the formulation is very important for its performance as it can be adversely affected by precipitation of the drug in an excipient matrix. Poor physical stability of the formulation can lead to phase separation of excipients that affects bioavailability, as well as therapeutic efficacy. Furthermore, the incompatibilities between formulation and gelatin shell of the capsule may cause brittleness, softness and delayed the disintegration or incomplete release of drug. The following cycles are carried out for these studies:

1. Heating cooling cycle: Six cycles of cooling and heating between refrigerator temperature (4°C) and elevated temperature (45°C) with exposure at each temperature for not <48 h are carried. Those formulations, which are stable, are then subjected to centrifugation test
2. Centrifugation: Formulations that pass the heating-cooling cycle are centrifuged at 3500 rpm for 30 min. Those formulations that does not show any phase separation are taken for the freeze-thaw stress test.
3. Freeze-thaw stress cycle: Three freeze-thaw cycles between 21°C and 25°C with storage at each temperature for not <48 h. Those formulations that pass this test show good stability with no phase separation, cracking or creaming. The formulations that pass this test are then further taken for dispersibility test for assessment of self-emulsification efficiency.[19]

In vitro dissolution technique

The quantitative *in vitro* dissolution studies are carried out to assess drug release from oil phase into aqueous phase by USP type II dissolution apparatus use of 900 ml of pH 1.2 phosphate buffer solution at 100 rpm and maintain the temperature at 37°C ± 0.5°C. Aliquots of 5 ml samples were withdrawn at regular intervals of time and volume withdrawn was replaced with fresh medium.[20] Samples taken were then analyzed by use of UV spectrophotometer at 313 nm.

RESULTS AND DISCUSSION

Screening of oils and surfactants

The results of the solubility of NVP inappropriate vehicles were showed in the Tables 1-3. From the solubility data; the oleic acid, tween 20, and PEG 600 were selected as oil, surfactant, and co-surfactant respectively.

Plot of pseudo ternary phase diagrams

Phase diagrams of the systems containing Oleic acid as an oil phase, Tween 20 as a surfactant and PEG 600 as a co-surfactant were constructed at the surfactant/co-surfactant (S/CoS) ratio of 1:4, 1:3, 1:2, 1:1 and 4:1 (w/w) to determine the existence of microemulsion region as showed in Figures 1-5, respectively. The phase study revealed that the obtained microemulsion regions at S/CoS ratios of 1:1 [Figure 1] was low, when compared with all other ternary plots. At S/CoS ratios of 1:2 [Figure 2], 1:3 [Figure 3], and 1:4 [Figure 4], an increase in the microemulsion regions gradually as concentration of co-surfactant increases, it indicates that the co-surfactant has some effect on the capability of forming micro emulsion. The ratio 4:1 [Figure 5] of S/CoS showed maximum microemulsion region when compared to all other ternary plots, which points that an increase in the concentration of surfactant gives the highest microemulsion regions among all other ternary plots. It indicates that the concentration of surfactant has a major effect on the microemulsion region forming capability of SEDDS.

Table 1: Solubility of nevirapine in different oils

Oil	Solubility in mg/ml ($\bar{X}$ ± SD)
Coconut oil	0.81±0.02
Arachis oil	1.823±0.031
Castor oil	6.093±0.021
Chaulmoogra oil	10.448±0.042
Olive oil	0.894±0.004
Sesame oil	6.455±0.012
Linseed oil	7.142±0.021
Oleic acid	20.49±0.04

SD: Standard deviation

Table 2: Solubility of nevirapine in different surfactants

Surfactants	Solubility in mg/ml ($\bar{X}$ ± SD)
Span 20	14.36±0.03
Span 80	14..43±0.041
Tween 20	17.35±0.02
Tween 80	10.63±0.04

SD: Standard deviation

Table 3: Solubility of nevirapine in different co-surfactants

Co-surfactants	Solubility in mg/ml ($\bar{X}$ ± SD)
PEG 200	7.47±1.02
PEG 400	8.04±0.82
PEG 600	8.98±1.24
PG	5.547±0.04

PEG: Polyethylene glycol, PG: Propylene glycol, SD: Standard deviation

From the observed experimental results, it was observed that the surfactant: co-surfactant ratios of 4:1 showed maximum microemulsion region when compared with all other ratios. Hence, S/Co-S ratio of 4:1 was selected for the formulation of SEDDS based on microemulsion region forming capability, and it was subjected to further studies.

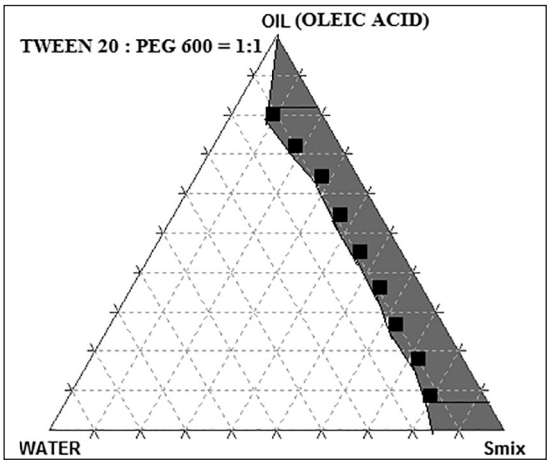

Figure 1: Ternary plot of Tween 20: Polyethylene glycol 600 = 1:1

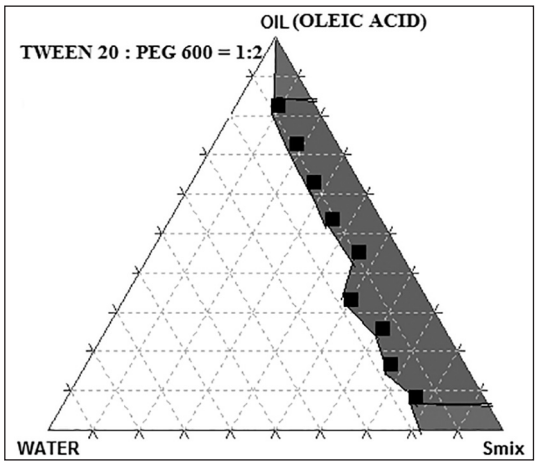

Figure 2: Ternary plot of Tween 20: Polyethylene glycol 600 = 1:2

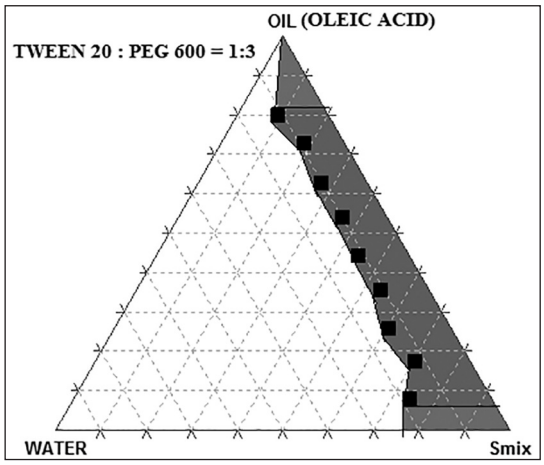

Figure 3: Ternary plot of Tween 20: Polyethylene glycol 600 = 1:3

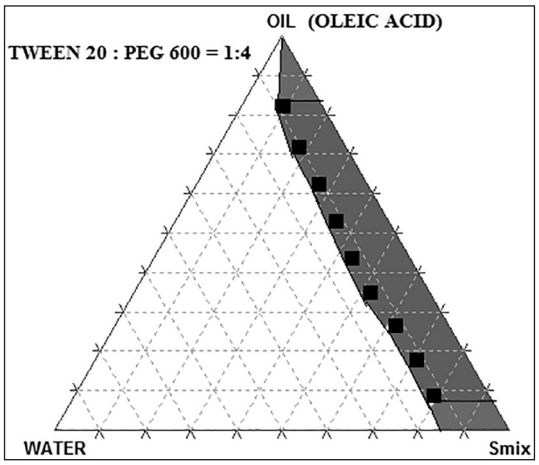

Figure 4: Ternary plot of Tween 20: Polyethylene glycol 600 = 1:4

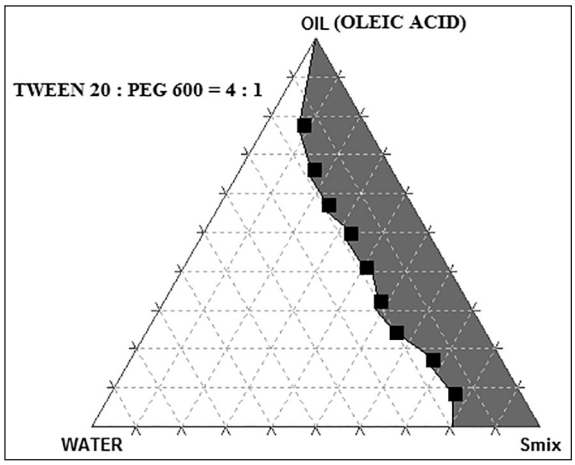

Figure 5: Ternary plot of tween 20: Polyethylene glycol 600 = 4:1

Formulation and optimization of nevirapine self-emulsifying drug delivery system by using 2^2 full factorial design

From the studies of pseudo ternary phase diagrams, it was found that the Surfactant: Co-surfactant ratio of 4:1 was showing large micro emulsion regions. And it was selected for formulation of SEDDS. In the 4:1 (S:Cs), we have 9 different ratios of S_{mix}: Oil, that is, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. In the listed ratios, the first two highest water consuming ratios are selected and are used for the formulation and optimization of NVP SEDDS by using 2^2 factorial design.[21] The results of Formulation and Optimization of NVP SEDDS by using 2^2 full factorial design were showed in Table 4.

Table 4: Composition of formulation

Component	F1	F2	F3	F4
Nevirapine (mg)	100	100	100	100
$S_{mixture}$ (%)	Low (48.3)	High (56)	Low (48.3)	High (56)
Oil (%)	Low (24)	High (32.2)	High (32.2)	Low (24)

Table 5: Drug content results

Formulation	Percentage of drug content ($\bar{X}$ ± SD)
F1	97.4±1.9
F2	98.2±0.8
F3	97.9±0.98
F4	98.9±0.7

SD: Standard deviation

Characterization of self-emulsifying drug delivery systems

Drug content

Assay of prepared NVP SEDDS was carried out by UV-visible spectrophotometer. A linear calibration curve was obtained at 313 nm in the range of (2-10 μg/ml) with a correlation coefficient (R^2) of 0.999. The Assay results are shown in Table 5.

The % drug content of all SEDDS formulations was found to be within the acceptable limits of drug content test.

Fourier transform infrared studies

The FT-IR studies were done to characterize the drug. Here, we are performing the FT-IR studies of the pure drug and best formulation (F4) to elicit interactions of drug with other excipients present in the formulation. The IR spectrum for the pure drug and SEDDS best formulation (F4) were given in Figures 6 and 7. The peak observed at 758.787 cm^{-1} is characteristic of the C-H bending of the aromatic group. The peak produced at 1289 cm^{-1} is characteristic of C=O stretching seen in alcohols. The peak observed at 1461 cm^{-1} is typical of C=C stretching of the aromatic group. The peaks observed at 1585 cm^{-1} and at 1643 cm^{-1} are characteristic of N=N and C=N stretching. The peak observed at 3184 cm^{-1} is characteristic of C-H alkene group present in the molecule. No interactions were detected between excipients and the NVP after observation of the spectrum of SEDDS best formulation, when compared with the pure drug spectrum.

Determination of droplet size and zeta potential

The charge of the droplets was determined by zeta potential measurement. Droplet size and the zeta potential of the formed emulsion were determined use of Zetasizer ZS 90 (Malvern Instruments, UK). Light scattering was monitored at 25°C at a 90° angle. The results of SEDDS formulations were listed in Table 6. From the data obtained through Figures 8 and 9, Table 6, it was found that the F4 formulation was best when compared with all other formulations and showed droplet size of 319.2 nm and zeta potential of −68.9 mV.

In vitro diffusion study

In vitro diffusion study was performed to compare the drug release from the developed NVP SEDDS. *In vitro* diffusion study of the NVP SEDDS (F1, F2, F3, and F4) was performed by using a dialysis technique. The drug release results of *In vitro* diffusion study were listed in Table 7 and Figure 10. After observation of the results, it was found that, nearly 55.634% ± 0.661% of drug was released from NVP SEDDS F4 formulation within 1 h compared with the other formulations, that is, F1, F2, and F3, which released 36.999% ± 0.012%, 49.422% ± 0.475%, and 43.211% ± 0.312%, of

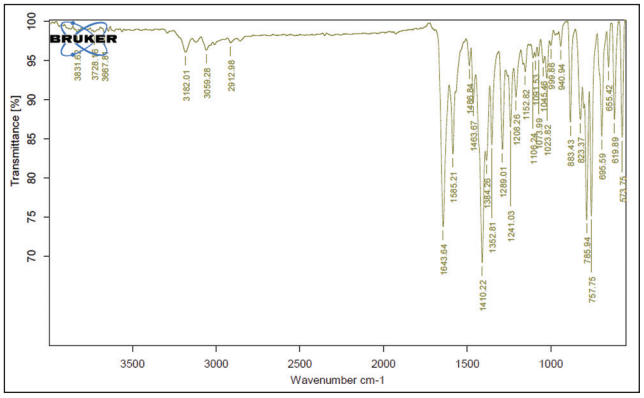

Figure 6: Fourier transform infrared graph of nevirapine pure drug

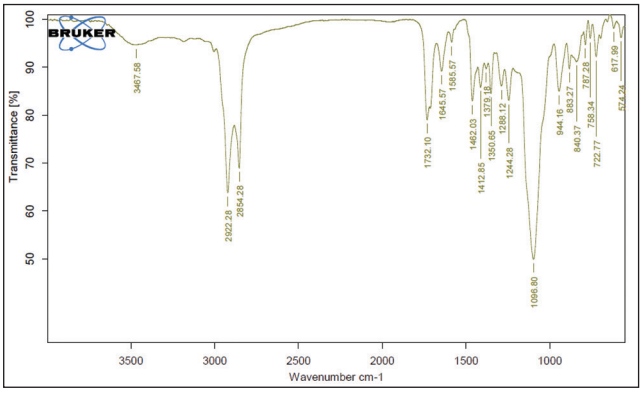

Figure 7: Fourier transform infrared graph of nevirapine self-emulsifying drug delivery system

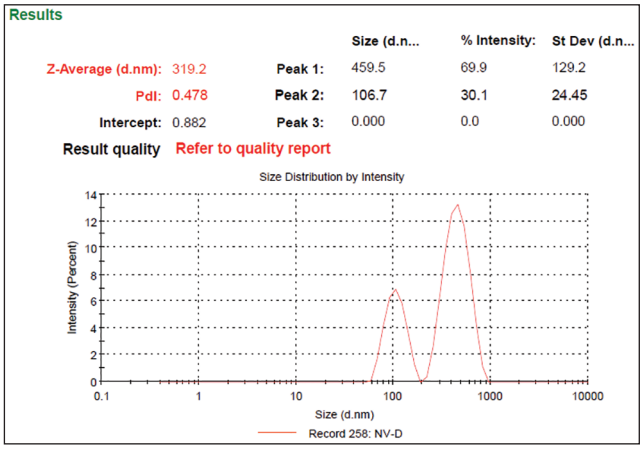

Figure 8: F4 Droplet size

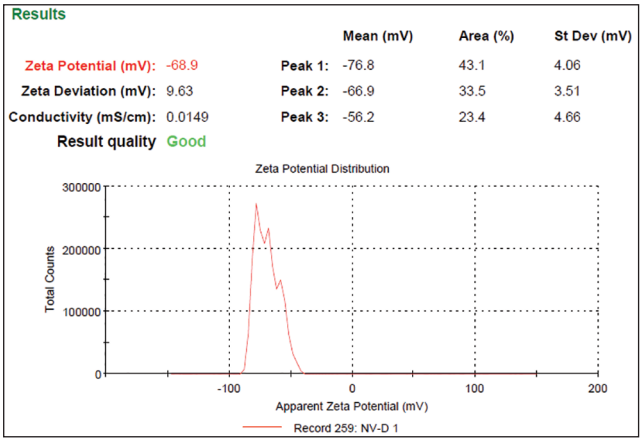

Figure 9: F4 Zeta Potential

Table 6: Zeta potential results of SEDDS formulations

Formulation code	Droplet size (nm)	polydispersibility index	Zeta potential (mV)
F1	1768	1.00	□39.9
F2	929.8	1.00	□40.5
F3	706.1	0.595	□71.5
F4	319.2	0.478	□68.9

SEDDS: Self emulsifying drug delivery systems

Table 7: *In vitro* diffusion data of the nevirapine SEDDS formulations in 0.1N HCl

Time (h)	Percentage of drug release			
	F1	F2	F3	F4
0	0	0	0	0
0.5	30.95±0.23	43.45±0.87	37.20±0.12	49.69±0.54
1	36.99±.012	49.42±0.47	43.21±0.31	55.63±0.66
1.5	42.96±0.51	55.32±0.65	49.14±0.22	61.50±0.59
2	48.87±0.05	61.15±0.72	55.01±0.33	67.29±.084
2.5	54.71±0.33	66.91±0.25	60.80±0.41	73.02±0.28
3	60.43±0.51	72.60±0.55	66.53±0.22	78.68±0.19
3.5	66.15±0.42	78.23±0.91	72.19±0.56	84.27±0.29
4	71.77±0.12	83.78±0.12	77.78±0.84	89.78±.016
4.5	77.33±1.21	89.27±0.123	83.30±0.51	95.23±0.12
5	82.81±0.56	94.68±0.09	88.75±0.32	98.24±0.03
5.5	88.23±0.23	97.67±0.04	94.13±0.12	
6	93.57±0.02		97.09±0.05	
6.5	96.52±0.08			

SEDDS: Self emulsifying drug delivery systems

the drug respectively. At the end of 5 h period, almost all the drug (98.245% ± 0.03%) diffused from the SEDDS F4 formulation compared with drug released from the other formulations, that is, F1, F2, and F3, which released 82.817% ± 0.56%, 94.685% ± 0.09%, and 88.751% ± 0.302% of the drug respectively [Figure 10].

It was found that, all the formulations followed first order kinetics as correlation (R^2) values of first order release kinetics were higher than that of zero order release kinetics showed in Table 8. The first order rate constant (K) was calculated from the slope of first order linear plot showed in Figure 11.

Thus, the drug release from the NVP SEDDS F4 formulation was found to be significantly rapid and higher as compared to that of the remaining SEDDS formulations. It could be suggested that the SEDDS F4 formulation resulted in a spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase. Thus, this greater availability of dissolved NVP from the SEDDS F4 formulation could lead to higher and rapid absorption and oral bioavailability.

Determination of self-emulsification time

Emulsification time is an important index for the assessment of the efficiency of emulsion formation. SEDDS should disperse

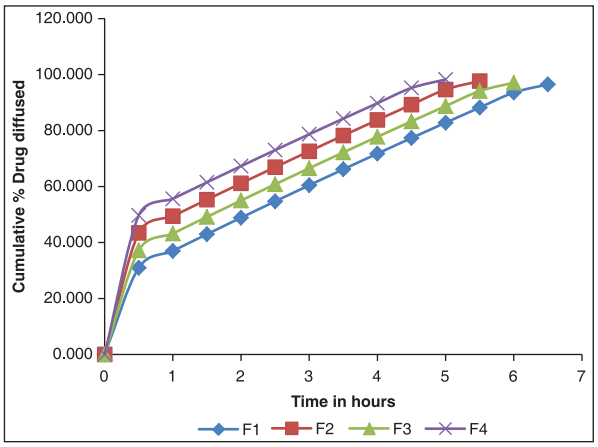

Figure 10: Comparative *in vitro* diffusion profile plot of different formulations of self-emulsifying drug delivery system in 0.1N HCl

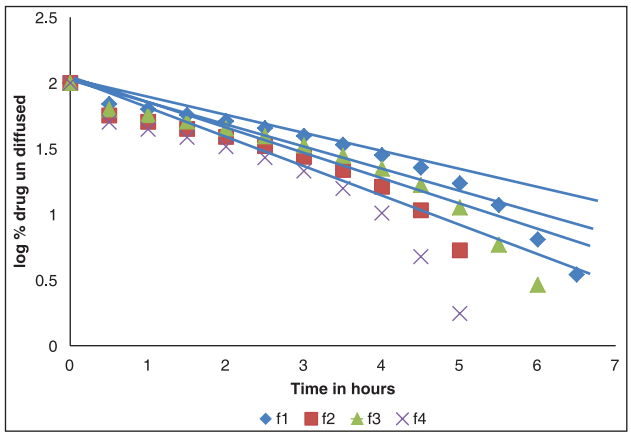

Figure 11: First order plot of diffusion data of all formulations

completely and rapidly when subjected to aqueous dilution under mild agitation. The emulsification time of all formulations was reported in Table 9.

After observation of Table 9, it was found that the F4 formulation forms microemulsion in a short time relatively among all other formulations which indicate that the F4 was best of all prepared formulations.

Rheological properties determination

The SEDDS systems were loaded in hard gelatin capsules. So SEDDS were easily pourable into capsules and such systems should not be too thick. The rheological properties (viscosity, flow) of the microemulsion are reported in Table 10.

From Table 10, it was found that F4 formulation was showing low viscosity and plastic flow, which indicates stability and pourability of formulation F4 was best among all other formulations.

Thermodynamic stability studies

The physical stability of the formulation is very important for its performance as it can be adversely affected by precipitation of the drug in an excipient matrix. Poor physical stability of the formulation can lead to phase separation of excipients that affects bioavailability, as well as therapeutic efficacy. Furthermore, the incompatibility between formulation and gelatin shell caused brittleness, softness and delayed the disintegration or incomplete release of drug. The following cycles were carried out for these studies, and the results were reported in the Table 11.

From Table 11, it was observed that, there were no appreciable changes in the formulations during stability studies, and hence it was concluded that the formulations are thermodynamically stable.

In vitro dissolution study

In vitro dissolution study was performed to compare the drug release from the developed NVP SEDDS formulations and pure drug. The quantitative *in vitro* dissolution studies is carried out to assess drug-release from the oil phase into the aqueous phase by USP type II dissolution apparatus. The results of

Table 8: *In vitro* diffusion kinetics of nevirapine SEDDS formulations in 0.1N HCl

Dissolution medium	Formulation code	Correlation co-efficient		*K* (min^{-1})	T_{50} (min)	T_{90} (min)
		Zero order	First order			
0.1N HCl	F1	0.8918	0.9251	0.0414	16.8	55.7
	F2	0.7539	0.9122	0.0574	12.1	40.1
	F3	0.8353	0.9478	0.0475	14.6	48.5
	F4	0.6693	0.9603	0.0705	9.8	32.6

SEDDS: Self emulsifying drug delivery systems

Table 9: Self emulsification time of all formulations

Formulation code	Self-emulsification time (s)
F1	135±20
F2	65±11
F3	90±15
F4	50±10

Table 10: Rheological properties of all formulations

Formulation code	Type of flow	Viscosity (cP)
F1	Plastic flow	1113
F2	Plastic flow	829
F3	Plastic flow	1775
F4	Plastic flow	476.3

Table 11: The observations of thermodynamic stability studies of all formulations

Formulation code	Heating cooling cycle	Centrifugation	Freeze thaw stress cycle
F1	No phase separation	No phase separation	No phase separation
F2	No phase separation	No phase separation	No phase separation
F3	No phase separation	No phase separation	No phase separation
F4	No phase separation	No phase separation	No phase separation

In vitro dissolution studies were listed in Table 12 and Figures 12 and 13 (first order plot). After observing the results, it was found

that, nearly 95.23% ± 0.13% of drug was released from NVP SEDDS F4 formulation within 45 min compared to the other formulations, that is, F1, F2, F3, and pure drug which released 77.332% ± 0.674%, 89.27% ± 0.268%, 84.137% ± 0.446% and 28.032% ± 0.395% of the drug respectively. At the end of 50 min period, almost all the drug (99.432% ± 0.03%) released from the SEDDS F4 formulation compared to drug released from the other formulations, that is, F1, F2, and F3, and pure drug which released 82.817% ± 0.124%, 94.685% ± 0.122%, and 89.7% ± 0.398% and 29.886% ± 0.639% of the drug respectively. Thus, the drug release from the NVP SEDDS F4 formulation was found to be significantly higher as compared to that of the remaining

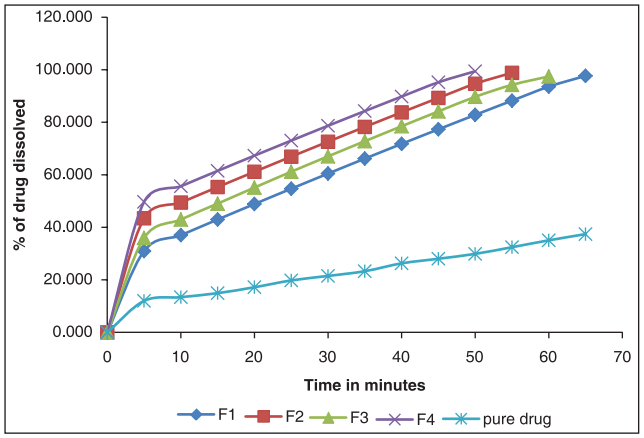

Figure 12: Comparative *in vitro* dissolution profile plot of different formulations of self-emulsifying drug delivery system and pure drug in 0.1N HCl

SEDDS formulations and pure drug. It could be suggested that the SEDDS F4 formulation resulted in a spontaneous formation of a microemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase. Thus, this greater availability of dissolved NVP from the SEDDS F4 formulation could lead to higher absorption and higher oral bioavailability.

It was found that, all the formulations followed first order kinetics as correlation (R^2) values of first order release kinetics were higher than that of zero order release kinetics. The first order rate constant (K) was calculated from the slope of first order linear plot showed in Figure 13. *In vitro* dissolution parameters such as, T_{50} (time required for dissolution of 50% of drug), T_{90} (time required for dissolution of 90% of drug), DE_{45} (Dissolution Efficiency), and correlation co-efficient values (first order) of all SEDDS formulations and pure drug are presented in Table 13.

By observing all the results, it implies that all the formulations of SEDDS were showing more drug release than pure drug. It indicates that the SEDDS formulations were helpful for the enhancement of solubility of NVP.

Among the four formulations of SEDDS and pure drug, F4 formulation offered the rapid release rate of NVP. The formulation F4 prepared with 56% of $S_{mixture}$ (surfactant [Tween 80] 44.8% + co-surfactant [PEG 600] 11.2%) and 24% of oil (oleic acid) was selected for further studies as it offered relatively rapid release of NVP when compared with other formulations used in this investigation.

Table 12: Comparison of dissolution studies of SEDDS formulations with pure drug

Time (min)	Percentage of drug dissolved				
	Pure drug	F1	F2	F3	F4
0	0.000	0.000	0.000	0.000	0.000
5	6.974±0.8	30.959±0.77	30.959±0.73	35.956±1.79	49.698±0.98
10	9.011±0.67	36.999±1.982	37.378±0.56	42.962±1.08	55.634±0.234
15	11.185±1.1	42.969±0.987	43.831±0.33	49.022±1.876	61.500±0.543
20	12.121±1.5	48.870±0.909	50.318±0.454	55.135±1.43	67.296±0.786
25	13.562±0.99	54.701±1.27	56.841±1.78	61.175±3.445	73.023±0.547
30	16.134±1.22	60.463±2.08	63.398±0.49	67.022±1.908	78.681±0.448
35	19.595±1.38	66.156±1.43	69.989±0.779	72.797±2.08	84.270±0.879
40	21.576±0.87	71.779±1.49	76.616±1.33	78.502±2.21	89.789±0.359
45	25.191±0.53	77.332±1.98	83.277±2.09	84.137±1.23	95.238±0.657
50	29.90±1.71	82.817±2.01	89.973±1.99	89.700±0.999	99.432±0.143
55	35.01±0.972	88.232±1.45	96.703±0.943	94.249±1.767	
60	39.87±0.47	93.577±0.92		98.503±1.879	
65	43.33±0.59	97.687±0.77			

SEDDS: Self emulsifying drug delivery systems

Table 13: *In vitro* dissolution kinetics of nevirapine SEDDS formulations and pure drug in 0.1N HCl

Dissolution medium	Formulation code	Correlation co-efficient		*K* (min^{-1})	T_{50} (min)	T_{90} (min)	% DE_{45} (%)
		Zero order	First order				
0.1N HCl	Pure drug	0.9114	0.9545	0.0075	92.8	308.2	18.03
	F1	0.8918	0.9251	0.0414	16.8	55.7	50.17
	F2	0.7539	0.9122	0.0574	12.1	40.1	61.73
	F3	0.8353	0.9478	0.0475	14.6	48.5	56.07
	F4	0.6693	0.9603	0.0705	9.8	32.6	67.5

SEDDS: Self emulsifying drug delivery systems, DE: Dissolution efficiency

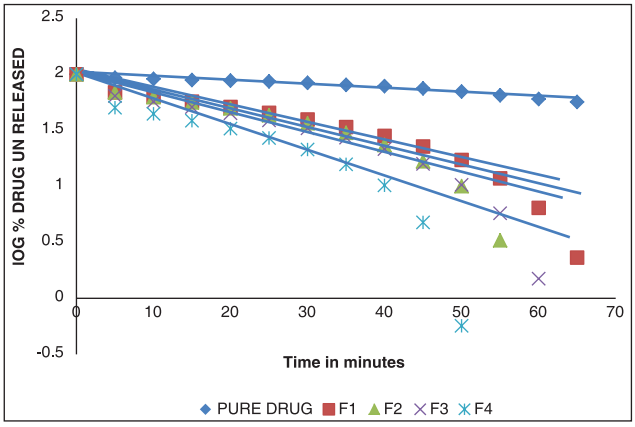

Figure 13: First order plot of dissolution data of all formulations and pure drug

CONCLUSION

The new emulsion formulations, that is, SEDDS are a promising approach for the formulation of NVP. The oral delivery of water-insoluble drugs like NVP can be made possible by SEDDS, which have been showed to be substantially improve oral bioavailability with future development of this technology. These current results demonstrated that SEDDS containing 24% w/w oleic acid oil (oil), 44.8% w/w, Tween 20 (surfactant) and 11.2% w/w PEG 600 (co-surfactant) was successfully developed with an increased solubility, increased dissolution rate of a poorly water-soluble drug, NVP when compared to all other formulations of SEDDS and pure form of the drug. The result from the thermodynamic stability studies confirms the stability of the developed formulation. Thus, the study confirms that the SEDDS of NVP can be used as a possible alternative drug delivery to traditional oral formulations of NVP with improved solubility and drug release.

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