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Research Article

Design and Evaluation of Self Nanoemulsifying Systems for Poorly Water Soluble HIV Drug

R. Panner Selvam*, P.K. Kulkarni

Department of Pharmaceutics, JSS College of Pharmacy, Mysore-15, Karnataka, India
Correspondence: selva.r9@gmail.com;*Tel.:*+91-9844795544

Abstract

Nevirapine is a potent; non-nucleoside reverse transcriptase inhibitor (NNRTI) used in combination with nucleoside analogues for treatment of Human Immunodeficiency Virus Type 1 (HIV-1) infection and AIDS. Nevirapine belongs to BCS class II which shows dissolution rate limited absorption. Self-nanoemulsifying systems (SNES) of nevirapine were prepared with the objective of improving the solubility and dissolution rate of the drug. Solubility of nevirapine was determined in many vehicles, which includes oils, surfactants and co surfactants. The efficient self emulsification region was determined by constructing pseudo-ternary phase diagrams. Based on the solubility studies, Captex 200 (Oil), Tween 20 (Surfactant) and Capmul MCM (Co surfactant) were selected for preparation of SNES. The prepared SNES systems were evaluated for thermodynamic stability, dispersibility, robustness to dilution, particle size measurements, zeta potential, refractive index, percent transmittance, viscosity, drug content and In vitro drug release. The FT-IR data rules out the interaction between the drug and the excipients. The DSC spectra confirm presence of drug in molecularly dissolved state in the vicinity of the lipid excipients. The optimized nevirapine SNES (G15) contains oil 15%, surfactant 22.5%, cosurfactant 7.5% which shows mean globule size of 52.7 nm. In vitro drug release from SNES was found to be 98.93 % in 30min whereas pure drug shows only 14.74% at the end of 30 min. The prepared SNES found stable after 3 month storing in stability chamber at 40°C and 75%RH.

Keywords: Nevirapine, self-nanoemulsifying systems, thermodynamic stability

1.Introduction

One of the world's leading causes of death with a major medical and economic impact on a society is AIDS, caused by the HIV virus. Around 36 million people are infected with human immunodeficiency type-1 (HIV-1) worldwide [1]. By introduction of highly active antiretroviral therapy (HAART) has brought significant reduction in mortality and conciliatory events, even in patients with very advanced stage of HIV infection [2]. The most important drug used in the HAART is nevirapine.

Nevirapine is a potent; non-nucleoside reverse transcriptase inhibitor (NNRTI) used in combination with nucleoside analogues for treatment of Human Immunodeficiency Virus Type 1 (HIV-1) infection and AIDS [3]. Nevirapine is a BCS class II compound with low aqueous solubility and high permeability, those posses the problem in the achievement of optimal dissolution kinetics from the developed dosage form [4]. Nevirapine shows characteristics of solubility rate limited absorption that cause decrease in bioavailability. Nevirapine is a weak base (pKa= 2.8) with low intrinsic water solubility (0.06 mg/ml) [5].

The techniques generally employed for solubilization of drug includes micronization, chemical modification, pH adjustment, solid dispersion, complexation, co - solvency, micellar solubilization, hydrotropy, etc [6]. Lipid emulsions are attractive systems for developing drug solubility of poorly soluble or practically insoluble drugs due to their capacity to integrate lipophilic drugs, for example, lipid solution, surfactant dispersion emulsion, liposomes, microemulsion, dry emulsion and self-microemulsifying formulations [7]. Self nanoemulsifying systems (SNES) are mixtures of oils and surfactants, ideally isotropic, and at times containing co-solvents/co surfactants, which emulsify instinctively to produce fine oil-in-water nanoemulsions when introduced into aqueous phase under gentle agitation. They are able to increase the bioavailability of poorly soluble drugs by bypassing the dissolution step and improving the permeability through biological membranes due to the presence of lipid and surfactant [8]. The study is aimed to develop nevirapine SNES, so that the solubility of the drug is improved which leads to better drug bioavailability.

2. Materials and methods

2.1. Materials

Nevirapine was provided as a gift sample from Strides Arco labs (Banglore, India). Labrafac PG, Labrafil were generous gift from Gattefosse, France (through Bombay College of Pharmacy, Mumbai). Capmul MCM, Captex 200 was a gift sample provided from Abitec Group, (USA). Span 80, Triethanolamine, PEG 800, PEG 200, Oleic acid, Castor oil were purchased from Merck (Mumbai). Tween 80, Tween 20, Ethyl oleate were purchased from Loba chemie pvt ltd, Mumbai. All other chemicals and buffers used were of analytical grade.

2.2. Solubility studies

Screening of excipients can be done by determining the equilibrium solubility of nevirapine in different oils and surfactants. Two ml of each of selected oil, surfactant sample was added in glass vial containing excess amount of nevirapine, the drug was mixed manually. After 48h these vials were centrifuged at 3000 rpm for 20 min and the amount of dissolved drug was determined by diluting the supernatant in methanol by UV- spectrophotometer at 282 nm [9].

2.3. Compatibility Study

Chemical interaction between the drug, lipid and surfactants were studied by FT-IR technique. It was scanned from 4000 to 400 cm-1 in a FT-IR spectrophotometer (FT-IR 8400S, Shimadzu, Japan). The IR spectrum of the physical mixture was compared with those of pure drug, lipid and surfactants and peak matching was done to detect any appearance or disappearance of peaks.

2.4. Construction of pseudoternary phase diagram

By employing water titration method, pseudo ternary phase diagrams of oil, surfactant/ cosurfactant (S/CoS), and water were developed [10-12]. From these, the extent of nanoemulsion region can be identified and its relation to other phases can be established. The pseudo-ternary phase plots were created by drop wise increment of distilled water to homogenous blend of oil, surfactant, and co-

surfactant, at ambient temperature. Based on the result of solubility studies Captex 200, Tween 20 and Capmul MCM were selected as oily phase, surfactant and co-surfactant, respectively.

The phase diagram were constructed at specific ratio of Smix (i.e. 1:0, 1:1, 1:2, 1:3, 1:4, 2:1, 3:1, and 4:1, w/w). Each liquid mixture was titrated with water and visually checked for phase clarity and flowability. By visual observation the following categories were accredited:

a) transparent and easily flowable: oil/water nanoemulsions

b) transparent gel: nanoemulsion gel

c) milky or cloudy: emulsion

d) milky gel: emulgel

Phase diagrams were then constructed using Chemix software.

2.5. Selection of formulations

Different formulations were selected from NE region of each phase diagram constructed so that drug could be incorporated into it on the following basis.

a) From each pseudo ternary phase diagram, different concentrations of oils were selected at a difference of 5% (10%, 15%, 20%, 25%, etc) from the NE region.

b) For each 5% of oil selected, the formula that used the minimum concentration of Smix for its NE formulation was selected from the phase diagram.

c) 50mg of nevirapine was selected as the dose for incorporation into SNES.

2.6. Evaluation of nanoemulsion

2.6.1. Thermodynamic stability tests

Thermodynamic stability studies are conducted to overcome the problem of selecting metastable formulation.

a) Centrifugation

Selected formulations from phase diagrams were centrifuged at 3500rpm for 30min and observed for phase separation, creaming and cracking. Those formulations which were stable were taken for heating cooling cycle.

b) Heating cooling cycle (H/C cycle)

Stability of SNES on variation of temperature was studied by H/C cycle. Six cycles between refrigerator temperature 4°C and 45°C with storage at each temperature of not less than 48h was studied. Those formulations, which were stable at these temperatures, were subjected to freeze thaw cycle.

c) Freeze thaw cycle

Three freeze thaw cycles between –21°C and +25°C with storage at each temperature for not less than 48 h was done for the formulations. Those formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility tests for assessing the efficiency of self emulsification [13].

2.6.2. Dispersibility tests

The efficiency of dispersibility of formulation was assessed using a standard USP XXII dissolution apparatus 2. One ml of each formulation was added to 500ml of water respectively at 37±0.5°C. A standard stainless steel dissolution paddle rotating at 50rpm provided gentle agitation .The in vitro performance of the formulations was visually assessed using the grading system as shown below.

Grade A: Rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance

Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance

Grade C: Fine milky emulsion that formed within 2min

Grade D: Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

Those formulations that passed the thermodynamic stability and also dispersibility tests in Grade A and B were selected for further studies.

2.6.3. Robustness to dilution

Robustness to dilution was studied by diluting it 50, 100 and 1000 times with various dissolution media viz. water and 0.1N HCl. The diluted nanoemulsions were stored for 12h and observed for any signs of phase separation or drug precipitation.

2.6.4. Particle Size Measurements

The mean globule size and polydispersity index (P.I.) of the resulting nanoemulsions were determined by photon correlation spectroscopy, which analyses the fluctuations in light scattering due to Brownian motion of the particles using a Zetasizer 3000 (Malvern Instruments Worcestershire, UK) Light scattering was monitored at 25ºC at a 90º angle.

2.6.5. Zeta potential determination

The emulsion stability is directly related to the magnitude of the surface charge. The zeta potential of the diluted SNES formulation was measured using a Malvern Zetasizer 3000. (Malvern Instruments Worcestershire, UK)

2.6.6. Viscosity

Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA, spindle # CPE40) was used to determine the viscosity of different formulations at 25±1°C at 10 rpm for 5min..

2.6.7. Refractive index and percent transmittance

The refractive index of the system was determined using Abbe refractometer by placing a drop of nanoemulsion on the slide. The percent transmittance of the system was measured using UV spectrophotometer (Shimadzu, Japan) keeping distilled water as blank.

2.6.8. Differential scanning calorimetry (DSC)

Thermograms of nevirapine and physical mixtures were obtained using DSC instrument (DSC-60, Shimadzu, Japan) equipped with an intracooler. The samples was hermetically kept in the aluminum pan and heated at constant rate 10°C/min, over a temperature range of 35°C to 210°C

2.6.9. Drug content estimation

Nevirapine was extracted from the SNES by dissolving in methanol later it was analyzed spectrophotometrically at 282 nm, against solvent blank.

2.6.10. Drug release studies

Drug release studies for nevirapine and nevirapine SNES were performed using dissolution apparatus II containing 900ml of 0.1N HCl as dissolution medium at 37±0.5°C. The speed of the paddle was adjusted to 50rpm. 50mg drug equivalent of the formulation (1ml) was directly introduced into the medium and a suitable aliquot (5ml) of sample was collected at 0, 5, 10, 15, 20, 30, 40, 60, 80, 160 min and with further dilutions the samples were analyzed spectrophotometrically at 282nm. An equivalent volume of fresh dissolution medium was added to compensate for the loss due to sampling [14].

2.6.11. Stability study

Optimized nevirapine SNES was sealed in ampoules and then placed in stability chamber which were maintained at 40°C/75%RH for 3 months. Duplicate samples were withdrawn at 0, 1, 2 and 3 months to evaluate their physical and chemical stabilities.

3. Results and Discussion

The drug solubility in various oils, surfactants is reported in Table 1. The excipients selected for the preparation of SNES should have the

ability to solubilize the drug at a high level in order to obtain a concentrated form that will be easy to load in the solid matrices [15].

Table 1. Solubility of nevirapine in various vehicles

Excipients	Solubility (mg/ml)
Ethyl oleate	122.2±3.28
Arachis oil	13.4±0.73
Castor oil	17.22±1.89
Oleic acid	17.97±1.10
Captex 200	215.8±2.64
Capmul MCM	164.57±1.78
Labrafac PG	184.93±1.41
Tween 20	79.64±0.97
Tween 80	67.92±1.04
PEG 200	48.02±0.94
PEG 800	46.42±0.75
Triethanolamine	58.94±1.26
SPAN 80	64.27±1.10
Water	0.08mg/ml

Data are mean ± SD, n=3

Because of poor solubility, stability problems and biocompatibility issues, the natural lipids were not selected in the formulation. Nevirapine showed highest solubility in Captex 200 (Oil), Tween 20 (Surfactant) and Capmul MCM (co-surfactant) than other oils and surfactants. Hence these excipients were selected for the preparation of SNES.

The characteristic peaks of nevirapine (amide group at 3097 cm^{-1} and 1640 cm^{-1} due to N - H and C=O stretching, respectively) were not affected and prominently observed in IR spectra of nevirapine along with other excipients as shown in Fig. 1. This clearly shows there is no interaction between drug and excipients.

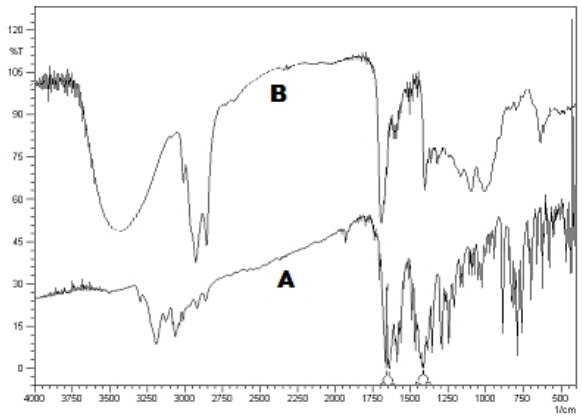

Fig. 1: IR spectra of nevirapine pure (A) and along with other excipients (B)

The concentration of the selected vehicles Captex 200, Tween 20 and Capmul MCM were optimized by pseudo-ternary phase diagrams. The phase diagrams for different oil-Smix-water systems were given in the Fig. 2. These phase diagrams shows only nanoemulsions region, to avoid the overcrowding of phase diagram. Use of non-ionic surfactants generally leads to less toxicity along with lower critical miceller concentration (CMC) as compared to their ionic

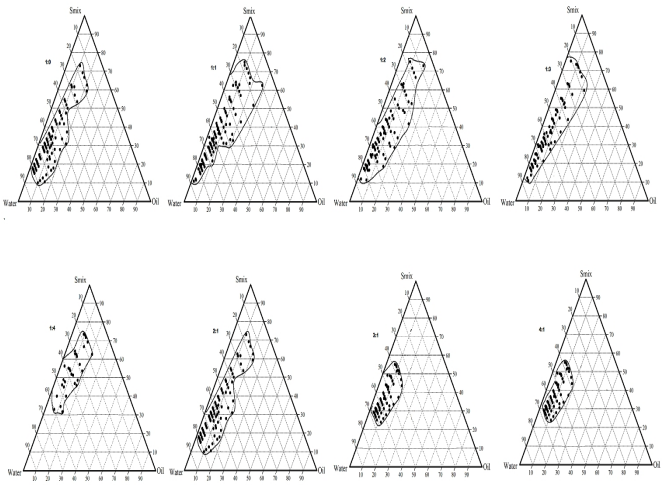

Fig. 2: Pseudoternary phase diagrams indicating the area of existence of o/w nanoemulsion containing different S_{mix}

Further, o/w nanoemulsions based on non-ionic surfactants are likely to offer better in vivo stability. In case of self emulsifying system without co surfactant, instantaneous formation of turbid gel was observed on addition to water. Therefore, this mixture without co-surfactant was considered as 'bad' emulsifying system as spontaneous emulsification was not observed. Transient negative interfacial tension and a fluid interfacial film are rarely achieved with the use of a single surfactant, usually necessitating the addition of a co-surfactant. The presence of co-surfactants decreases the bending stress of the interface and allows an interfacial film with sufficient flexibility to assume different curvatures required to form a nanoemulsion over a wide range of compositions. Pseudo ternary phase diagrams depicts that oil could be solubilized upto the extent of 29% (w/w). Therefore, from each phase diagram different concentrations of oil that formed a nanoemulsion was selected at 5% intervals (10%, 15%, 20% and 25%). So that, largest number of formulations could be selected covering the nanoemulsion are of the phase diagram (Table 2).

Table 2. Results of thermodynamic stability test and dispersion test

S_{mix}	Oil	S_{mix}	Aqueous	Centrifuge	H/C cycle	Freeze Thaw	Disperse Grade	Inference
1:0 (A)	10	8	82	Pass	Fail	Fail	D	Fail
	15	10	75	Pass	Fail	Fail	D	Fail
	20	18	62	Pass	Pass	Fail	D	Fail

S_{mix}	Oil	S_{mix}	Aqueous	Centrifuge	H/C cycle	Freeze Thaw	Disperse Grade	Inference
1:1 (B)	10	20	70	Pass	Fail	Fail	A	Fail
	15	30	55	Pass	Pass	Pass	A	Pass
	20	29	51	Pass	Fail	Fail	A	Fail
	25	40	35	Fail	Fail	Fail	A	Fail
1:2 (C)	10	11	79	Pass	Fail	Fail	A	Fail
	15	15	70	Pass	Pass	Fail	A	Fail
	20	29	51	Pass	Fail	Pass	A	Fail
	25	40	35	Pass	Fail	Fail	A	Fail
1:3 (D)	10	18	72	Pass	Fail	Fail	A	Fail
	15	30	55	Pass	Fail	Fail	A	Fail
	20	26	54	Pass	Pass	Pass	A	Pass
1:4 (E)	10	30	70	Pass	Pass	Pass	A	Pass
	15	30	55	Pass	Fail	Fail	A	Fail
	20	57	23	Fail	Fail	Fail	A	Fail
2:1 (F)	10	9	81	Pass	Fail	Fail	A	Fail
	15	10	75	Pass	Fail	Fail	A	Fail
	20	18	62	Fail	Fail	Fail	A	Fail
3:1 (G)	10	21	79	Pass	Fail	Fail	A	Fail
	15	30	55	Pass	Pass	Pass	A	Pass
	20	40	40	Pass	Fail	Fail	A	Fail
4:1 (H)	10	22	68	Pass	Fail	Fail	A	Fail
	15	25	60	Pass	Pass	Pass	A	Pass
	20	40	40	Pass	Fail	Fail	A	Fail

For each percentage of oil, only those formulations were taken from the phase diagram which used minimum concentration of S_{mix} [16]. These optimized Oil and S_{mix} concentrations were mixed gently for homogeneity of the formulation. On the basis of the thermodynamic stability studies and dispersibility tests, it was found that five formulations were passed and selected for further characterization (Table 3).

Table 3. Optimized formulations selected from phase diagram that passed thermodynamic stability test and dispersion test

Formulation Code	S_{mix} ratio	Oil %	Surfactant %	Co-Surfactant %	Aqueous %
B15	1:1	15	15	15	55
D20	1:3	20	6.5	19.5	54
E10	1:4	10	6	24	70
G15	3:1	15	22.5	7.5	75
H15	4:1	15	20	5	60

Formulations taken from ternary phase diagram (o/w nanoemulsion region) were subjected to thermodynamic stability and dispersibility tests in order to remove metastable formulations in minimum possible time. The formulations which passed thermodynamic test and dispersibility test were presented in Table 3 along with their concentrations. The optimized formulations B15, D20, E10, G15 and H15 were robust to all dilutions and did not show any phase separation or precipitation. The sequence of viscosity of prepared SNES batches is as follow G15>H15>B15> D20> E10 (Table 4).

Table 4. Data of particle size, zetapotential, polydispersibility index, Viscosity, Refractive index (R.I) and % transmission

Code	Particle Size (nm)	Zeta potential (mV)	Poly-dispersibilty index	Viscosity (cps)	Refractive index	% Transmission
B15	128.7	-5.42	0.525	27.95±0.17	1.422±0.3	98.74±1.26
D20	97.45	-5.87	0.473	24.37±0.28	1.436±0.22	98.59±1.47
E10	78.32	-6.45	0.421	21.22±0.17	1.431±0.31	98.26±1.19
G15	52.7	-7.73	0.164	36.76±0.37	1.459±0.26	97.64±2.4
H15	67.8	-6.94	0.248	32.64±0.28	1.456±0.21	97.94±1.96

Data are mean ± SD, n = 3

The viscosity results show that concentration of surfactant is directly proportional to the viscosity. The mean particle sizes of the optimized diluted SNES are shown in Table 4. The formulation G15 shows the lowest particle size when compare to the others, this is because of its low concentration of oil and high concentration of surfactant [17]. Several research studies have reported that the zeta potential played an important role in the interactions with mucus membrane of the gastrointestinal tract. Due to slightly negative charge of the droplet, aggregations will not take place. Because the droplets have a lower negative potential, they are likely to enhance the intestinal absorption of nevirapine. The refractive index of the SNES was similar to that of refractive index of the water (1.333). In addition, the developed system showed percent transmittance >97%. The refractive index and percent transmittance data confirms the transparency of the system. Drug content of all the optimized formulations was found to be more than 98%.

DSC curves of pure drug, physical mixture were shown in Fig. 3. Pure drug show sharp endothermic peak at near about 197.87ºC, which may be melting point of drug. The physical mixture did not show any obvious peak for nevirapine, may be due to more dilution of the drug in the excipients or absence in the sample exposed to DSC [5].

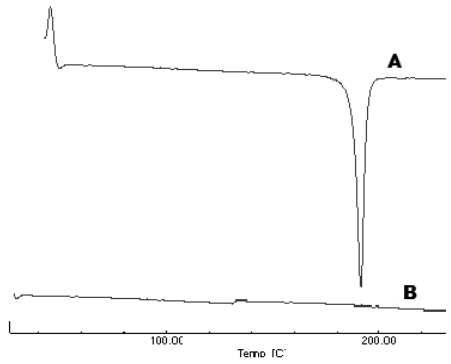

Fig. 3: DSC spectra for pure drug (A) and formulation (B)

The in vitro drug release studies were performed in order to ensure the fast release of the nevirapine to the dissolution medium. Furthermore, it also gives an idea about the emulsification efficiency of the developed system. Surfactant molecules in the oily solvent create a system of reverse micelles, with the hydrophilic inner core and the external layer formed by hydrophobic groups of the surfactant in the oily medium [18]. Upon mild agitation followed by dilution in aqueous medium, the reverse micellar solution undergoes transformation into a liquid crystalline system [19]. The amount of water solubilized by the reverse micelles depends on the type and concentration of surfactant, type of oil, temperature and co-solvent concentration. The *in vitro* drug release profile of B15, D20, E10, G15, H15 and pure drug were evaluated in 0.1N HCl. It was observed that all the SNES; B15, D20, E10, G15 and H15 released more than 80% of drug within 60 min (Fig. 4).

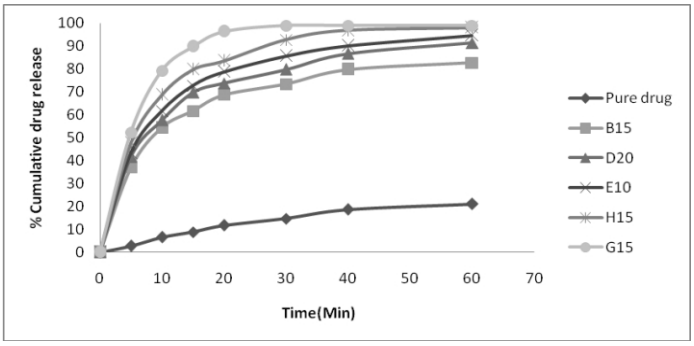

Fig. 4: Comparative dissolution profile of nevirapine and its SNES

Of the three formulations, G15 shows the fastest release which is due to the less oil concentration in its composition; whereas the pure drug shows only the release of 21.05% at 60 min. It is theorize that the oil/S_{mix} and water phases effectively swell, decrease the oil droplet size and eventually increase the release rate. This improved availability of dissolved nevrapine from the SNES could lead to increased absorption and higher oral bioavailability. The stability study of the optimized formulation (G15) stored at 40ºC and 75%

Table 5. Stability study data of optimized formulation (G15) under storage condition of 40°C/75% RH

Testing interval	Description of Formulation	FT-IR Study	Drug content	% cumulative drug release at 30min
Initial (0 time	Transparent	Complies	98.74±0.41	98.73±0.24
1 month	Complies	Complies	98.66±0.27	98.67±0.48
2 month	Complies	Complies	97.54±0.57	97.98±0.21
3 month)	Complies	Complies	97.33±0.36	97.21±0.36

No significant change in the appearance and content was observed during the study period. The FT-IR data confirms no change in the drug occurs throughout the study. The cumulative % drug release behavior of the optimized formulation was remained unchanged during storage.

4. Conclusion

The summary of present research work concludes that SNES for lipophilic nevirapine was successful developed using Captex 200 (Oil), Tween 20 (Surfactant) and Capmul MCM (Co surfactant). The optimized SNES consists of oil 15%, surfactant 22.5%, co-surfactant 7.5% which had sufficient drug loading, rapid emulsification in aqueous media and producing droplet size in the range of nanoemulsion. The solubility of nevirapine was significantly improved and thus overcomes dissolution rate-limited absorption of nevirapine.

Conflict of Interest

We declare that we have no conflict of interest

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