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Self-Microemulsifying Drug Delivery Systems (SMEDDS) of Efavirenz: Formulation Design, *in Vitro* and *in Vivo* Assessment

Mahendra Nakarani[1], Jayvadan Patel[2], Priyal Patel[1], Subhash Vaghani*[3]

[1]S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Gujarat, India.
[2]Nootan College of Pharmacy, Visnagar, Gujarat, India.
[3]ROFEL, Shri G. M. Bilakhia College of Pharmacy, Vapi, Gujarat, India.

Abstract
Efavirenz SMEDDS formulations containing lipid, surfactant and cosurfactant were developed and evaluated *in vitro* and *in vivo*. Pseudo-ternary phase diagrams composed of lipid, cosurfactant, surfactant and water were mapped and the region of microemulsion occurring was plotted. SMEDDS formulations were investigated for microemulsifying properties, clarity, precipitation, % Transmittance, Viscosity, Droplet size/size distribution, Zeta potential and long-term physical stability. The morphology of efavirenz microemulsion was observed by transmission electron microscopy (TEM). The *in vitro* release profiles of SMEDDS were compared with the release profiles of efavirenz from the conventional tablet and pure efavirenz. The pharmacokinetic study was performed by oral administration of 4.465 mg/kg efavirenz to Albino rats in different formulations. The oral bioavailability of efavirenz in SMEDDS capsules was significantly increased than that of the conventional tablet ($p<0.05$). The study confirmed that SMEDDS formulation can be used as a possible alternative to traditional oral formulations of efavirenz to improve its bioavailability.

Keywords: Efavirenz, self-microemulsifying drug delivery systems, bioavailability, pseudo ternary phase diagram, dissolution.

Introduction

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 (HIV-1). Its activity is mediated predominantly by noncompetitive inhibition of HIV-1 reverse transcriptase (RT). It is practically insoluble in water (<10 μg/mL). Efavirenz is used in combination with other anti-retroviral agents for the treatment of HIV-1 infection in children and adults (Montgomery et al. 2001). Its long half-life allows once-daily dosing and therefore presents an advantage for treatment compliance and efficacy (Gazzard 2000). It undergoes extensive metabolism, mainly by the cytochrome P-450 isoenzyme, CYP2B6, which is known to exhibit extensive inter-individual variability. This could lead to heterogeneity in response to treatment. In addition, differences in efavirenz pharmacokinetics between various racial/ethnic groups have been reported (Hass et al. 2004, Barrett et al. 2002, Fletcher 1999).

According to Biopharmaceutics Classification System (Amidon et al. 1995), Efavirenz belongs to Class II drugs and its solubility is too low to be consistent with complete absorption, even though it is highly membrane permeable.

***Corresponding author:** subhashvaghani@gmail.com

Correlation of *in vivo* results with dissolution tests is likely to be best for Class II drugs, because in this case the dissolution rate is the primary limiting aspect to absorption (Six 2004). Many approaches to improve oral bioavailability have been researched, such as salt forming techniques, complexation (i.e. cyclodextrins), particle size reduction, solubilization based on cosolvent or surfactant, etc.(Shen et al. 2006) for class II drugs.

SMEDDS are defined as isotropic mixtures of oil, surfactant, cosurfactant and drug that rapidly form o/w microemulsion when exposed to aqueous media under conditions of gentle agitation or digestive motility that would be encountered in GI tract (Borhade et al. 2008).

Self-microemusifying drug delivery systems (SMEDDS) have received great attention recently for its potential in improving oral bioavailability for the delivery of poorly water soluble drugs (Shen et al. 2006).

SMEDDS formulation is composed of lipid, surfactant and cosurfactant, with or without water. The system has the ability of forming oil-in-water (o/w) microemulsion when dispersed by aqueous phase under gentle agitation. The agitation required for self-emulsification comes from the digestive motility provided by the movement of stomach and intestine in the gastrointestinal tract (Shen et al. 2006). SMEDDS presents the drug in nanosized droplets offering large interfacial area for drug diffusion (Borhade et al. 2008, Gursoy et al. 2004, Lawrence et al. 2000, Pouton 2000). Furthermore, SMEDDS offer various advantages such as reduction in inter and intra subject pharmacokinetic variability, improvement in lymphatic transport and GI permeability and reversal of P-gp efflux, all of which help in improving bioavailability of hydrophobic drugs (Borhade et al. 2008).

Furthermore, since drugs can be loaded in the inner-phase and delivered by lymphatic bypass share, SMEDDS protect drugs against hydrolysis by enzymes in the gastrointestinal tract and reduce the presystemic clearance in the gastrointestinal mucosa and hepatic first-pass metabolism (Shen et al. 2006). The principle of self-emulsification is still the subject of speculation. Various models, and speculation about the absorption in the gastrointestinal tract, have been proposed to improve bioavailability of water-insoluble compounds caused by lipidic excipients, such as altering the gastrointestinal motility, increasing bile flow and mesenteric lymph flow, etc. (MacGregoret al. 1997, Charman 2000, Agoram 2001, Shen et al. 2001, Wagner et al. 2001). Lipid-based drug delivery systems have been developed to overcome the possible adverse influence of P-glycoprotein (Porter 2001). On all accounts, SMEDDS can improve oral bioavailability significantly.

The objectives of this study were to develop and characterize the optimal formulation of SMEDDS containing efavirenz to enhance the solubility, dissolution and assess bioavailability compared with conventional marketed formulation (Efavir® tablet) using animal model.

Materials and Methods

Efavirenz was supplied by Cipla Ltd. India. Efavir (Cipla Ltd. India) was purchased from market. Labrafil M 2125 CS, Lauroglycol 90, Caproy 90, Labrafac Lipophile WL 1349, Labrafil M 2130 CS, Labrafac PG, Transcutol HP, Gelucire 44/14 and Labrasol were supplied by Gattefosse Co. (France). Cremophor EL was supplied by BASF Co. (Germany). Capmul MCM C8, Campul MCM C10, Captex 200 and Caprol PGE 860 were supplied by Abitech Corporation, USA. Tween 80, Tween 20, Glycerol, PEG 400, propylene glycol, Soyabean Oil, Safflower Oil and Ammonium acetate AR grade were purchased from S.D. Fine Chemicals, Mumbai, India. Atorvastatin calcium was supplied by Cadila Healthcare. Hard gelatin capsules were obtained as gift samples from associated capsules, Mumbai,

India. Acetonitrile (HPLC grade) was purchased from Qualigens. Nylon syringe filters (0.45 μm) was procured from Millex-HN, Millipore (Mumbai, India). Distill water used through out study. All other chemicals and solvents were of analytical grade.

HPLC Analysis of Efavirenz

A high performance reverse phase HPLC method was developed for analysis of Efavirenz. The HPLC apparatus consisted of Waters liquid chromatograph 2707 Autosampler, equipped Waters-2489 UV/Visible detector and dual channel with empower software. The column used was stainless steel (25 cm x 4.6 mm i.d. C_{18} column), 5 μm particle size, make: Inertsil ODS 3V. Chromatographic analysis was carried out at ambient temperature. The Efavirenz was separated isocratically with a mobile phase consisting of solution A (Dissolved 800 mg ammonium in 1 L of water and pH was adjusted to 7.5 using 2 % v/v ammonium solution) and solution B (Acetonitrile) in the volume ratio of (40:60 v/v). The mobile phase was filtered and degassed prior to use. The flow rate was kept 1.5 mL min^{-1} with an injection volume 20μL. Column oven temperature was maintained at 25°C and detection wavelength was set at 252 nm. The retention time of efavirenz was 15.5 mins. The developed method was validated in terms of linearity, accuracy, precision, system suitability, limit of detection and limit of quantitation.

Solubility of Efavirenz

The solubility of Efavirenz in various components (oils, surfactants and cosurfactants) was determined as follows: Five hundred mg of each of the selected vehicle was added to each cap vial containing an excess of Efavirenz. Mixing of the systems was performed using a vortex mixer (Remi, India) to facilitate the solubilization. Formed suspensions were then shaken with a shaker at 25°C for 48 hours. After reaching equilibrium, each vial was centrifuged (Remi, India) at 3000 rpm for 10 minutes and excess insoluble Efavirenz was discarded by filtration using a membrane filter (0.45 μm, 13 mm, Whatman, Mumbai, India). The concentration of Efavirenz was then quantified by HPLC.

Self Emulsification Studies

Self-emulsification ability of various surfactants and cosurfactants were screened by mixing selected surfactants with selected co-surfactants in 1:1 w/w ratio to select the best surfactant and cosurfactant combination. Oily phase was added to this mixture in 1:3 (w/w), heated at 40–50 °C and vortexed to form homogenous mixture. Ratio of oil to surfactant was decided on the basis of requirements stated by Pouton (Pouton 2000) for spontaneously emulsifying systems and represents Type III system. Approximate 500 mg of oil-surfactant and co-surfactant mixture dispersed into 250 ml of double distilled water in a glass beaker with gentle stirring. Visual test was used to assess self-emulsification in terms of dispersability, ease of emulsification and final appearance using grading system (Table 1) (Borhade 2008).

Table 1. Visual assessment of efficiency of self-emulsification

Dispersibility and appearance	**Time of self emulsification**	**Grade**
Rapid forming microemulsion which is clear or slightly bluish in appearance	<1 min	I
Rapid forming, slightly less clear emulsion which has a bluish white appearance	<2 min	II
Bright white emulsion (similar to milk in appearance)	<3 min	III
Dull, greyish white emulsion with a slightly oily appearance that is slow to emulsify	>3 min	IV
Exhibits poor or minimal emulsification with large oil droplets present on the surface	>3 min	V

Pseudo-ternary Phase Diagrams

The pseudo-ternary phase diagrams of oil, surfactant/co-surfactant (S/CoS) and water were developed using water titration method (Borhade 2008). Three types of non-ionic surfactants, namely Cremophore EL, Tween 80 and Gelucire 44/14, were combined with 2 types of solubilizers as cosurfactants (Labrasol and PEG 400). A lipid employed was Labrafac lipophile WL 1349. Surfactant was blended

with cosurfactant in the ratio of 1:0.5, 1:1, 1:2, and 1:3 (i.e. S/CoS, w/w). Volumes of each surfactant and cosurfactant mixture (S/CoS) were blended with lipid in a ratio of 9.5:0.5, 9.0:1.0, 8.5:1.5, 8.0: 2.0, 7.5:2.5, 7.0 :3.0, 6.5:3.5, 6.0: 4.0 , 5.5: 4.5, 5.0:5.0, 4.5: 5.5, 4.0: 6.0, 3.5 : 6.5, 3.0: 7.0 , 2.5 : 7.5, 2.0: 8.0, 1.5: 8.5 to 1:9 (w/w). Then each mixture was titrated with water and visually observed for phase clarity. The concentration of water at which turbidity-to-transparency and transparency–to-turbidity transitions occurred was derived from weight measurements. After the identification of microemulsion region in the phase diagrams, the microemulsion formulations were selected at desired component ratios. In order to form the microemulsion, a series of SMEDDS were prepared. Phase diagrams were then constructed using Sigma plot 11® software.

Preparation of SMEDDS Formulations

After the pseudo-ternary phase diagrams were plotted and compared, optimal surfactant, co surfactant and lipid combinations were selected. Efavirenz SMEDDS formulations were prepared by dissolving Efavirenz into mixture of Oil, Surfactant and co surfactant in a glass beaker, heated at 40-50°C to form homogenous mixture and stored at room temperature until used. Various formulations were designed as shown in Table 2 to optimize the formulation.

Table 2. Various formulae for optimization of SMEDDS

Name of Ingredients	EF1	EF2	EF3	EF4	EF5	EF6
Efavirenz (mg)	50	50	50	50	50	50
Labrafac Lipophile WL 1349 (mg)	150	125	100	150	125	100
Tween 80 (mg)	287.5	300	312.5	237.5	250	287.5
Labrasol (mg)	287.5	300	312.5	-	-	-
PEG 400 (mg)	-	-	-	237.5	250	287.5
Total weight (mg)	775	775	775	675	675	675

Self-Emulsification and Precipitation Assessment

Evaluation of the self-emulsifying properties of SMEDDS formulations was performed by visual assessment as previously reported (Khoo et al. 1998). In brief, different compositions were categorized on speed of emulsification, clarity, and apparent stability of the resultant emulsion. Visual assessment was performed by drop wise addition of the preconcentrated (SMEDDS) into 250 mL of distilled water and 0.1 N HCl. This was done in a glass beaker at room temperature and the contents were gently stirred magnetically at ~100 rpm. Precipitation was evaluated by visual inspection of the resultant emulsion after 48 hours. The grading system used was as per given in Table 1.

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 (Remi, India) was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations stable to phase separation were selected for further studies.

Viscosity

The viscosity of the prepared SMEDDS formulations was determined without dilution by Brookfield LVT (USA) using spindle # 2 with speed 12 rpm at 25 ± 0.5°C. Viscosity of SMEDDS formulation was calculated using below equation;

$$\text{Viscosity (Cps or mPas)} = \text{Dial reading} \times \text{Factor}$$

Percentage transmittance

Percentage transmittance of the prepared SMEDDS formulations was determined spectrophotometrically using UV–Visible spectrophotometer (UV-1800 240 V, Shimadzu, Japan). One

ml of the formulation was diluted to 50 times and 100 times using distill water and 0.1 N HCl and analyzed at 650 nm.

Droplet size/Polydispersibility index (PDI) and Zeta potential

The mean droplet size and polydispersity index (PDI) were calculated from intensity, volume, number and modal distribution assuming spherical particles. The droplet size of the SMEDDS formulations was measured by Zeta Sizer 3000 (Malvern Instrument, UK). For measurement of droplet size, 1 ml samples were diluted with 250 ml of distilled water and 0.1 N HCl to diminish droplet interactions. Droplet size/size distribution and Zeta potential was measured by putting sample in zeta sizer cell at 25°C.

Transmission Electron Microscopy (TEM)

The morphology of SMEDDS was observed by transmission electron microscope (TEM) (PHILIPS TECNAI 20, Holland). SMEDDS was diluted with distilled water 1:25 and mixed by slightly shaking. Then, a drop of sample obtained after dilution was placed on copper grids. The excess was drawn off with filter paper. TEM was conducted with negative staining of phosphotungstic acid (PTA) solution (1%, w/v) and dried in air at room temperature before loading in the microscope.

In Vitro Dissolution Studies

The quantitative *in vitro* release test was performed using dissolution test USP model: TDT-08L (Electrolab, India) apparatus II at 50 rpm. The SMEDDS formulations were kept into hard gelatin capsules and used for drug release studies. Nine hundred mL of distilled water, 0.1 N HCl (pH 1.2) and acetate buffer (pH 4.5) were taken as dissolution mediums and each were containing 1.0 % SLS. A 5 mL sample of sample was taken out at 10, 15, 30, 45 and 60 min time intervals and subjected to drug analysis using HPLC method. The removed volume was replaced each time with 5 mL of fresh medium. Results were compared with those of plain Efavirenz and marketed formulation (Efavir®, Cipla ltd).

Pharmacokinetic Study in rats

Albino rats were used for the pharmacokinetic study. They were kept under standard laboratory conditions, temperature at 25 ± 2 °C and RH of 55 ± 5%. Animals were divided into three groups with three animals in each group and each group was given a different Efavirenz formulation EF2, EF5 and marketed formulation (Efavir, Cipla ltd). All the studies were carried out as per the local licensing authority (NPC/CPCSEA/22/2009) at Nootan Pharmacy College, Visnagar, Gujarat, India. The formulations were given orally using 18-gauge oral feeding needle. Dose for the rats was calculated based on the body weight of rats according to the surface area ratio (Paget and Barnes, 1964) which showed that 200 gm rat required 0.893 mg drug. The rats were anaesthetized and blood samples were withdrawn from retro-orbital plexus of the rats. For zero time analysis, blank blood sample was withdrawn before administration of drug. And after administration, blood samples were collected after 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0 and 48.0 hour time interval into sodium citrate containers. During the collection of the blood, the blood was mixed thoroughly to prevent clotting of the samples. The sodium citrate blood samples were first centrifuged at 2500 rpm for 15 minutes using a Remi centrifuge. The supernatant containing plasma was then separated using micropipette. Hundred µl of plasma was taken in a 2 ml micro centrifuge tube and 100µl of Acetonitrile was added into it to precipitate the proteins present in it. The tube was vortexed for 1 min followed by centrifugation at 2500 rpm for 15 min. Atorvastatin calcium was added as an internal standard, the supernatant was taken and 20 µl was injected into Shimadzu HPLC system after filtration.

HPLC for animal study

The mobile phase consisted of 10mM phosphate buffer pH 2.4 (adjusted with 1N HCl) and acetonitrile (55:45, v/v). Prior to preparation of the mobile phase, the phosphate buffer and acetonitrile were degassed separately using a vacuum pump. The analytical column was a (C_{18}, 150 mm × 4.6 mm ID, 5µ particle size (Lichrospher 100 RP-18e, Merck, Germany). The UV detector was set at 245 nm. The

chromatogram was run for 15 min at a flow rate of 2.0 ml/min at ambient temperature. Efavirenz and Atorvastatin calcium was separated at 12.62 mins and 8.0 mins respectively.

Maximum whole blood concentration (Cmax) and time to achieve Cmax (Tmax) were calculated from concentration time curve data. The area under the concentration time curve ($AUC_{0\rightarrow48}$) was calculated to the last blood concentration and extrapolated to infinity ($AUC_{0\rightarrow\infty}$). These parameters and other pharmacokinetic parameters were calculated by using QUICK CALC software.

Stability study

Chemical and physical stability of Efavirenz SMEDDS formulations was assessed at 40±2 °C/75±5% relative humidity (RH) as per ICH Guidelines. SMEDDS formulation EF2 was filled in size '00' hard gelatin capsules, while SMEDDS formulation EF5 pack in glass vials due to PEG 400 is reported to be incompatible with hard gelatin capsules and stored for 3.0 months. Samples were charged in stability chambers (Thermolab, Mumbai, India) with humidity and temperature control. Samples were analyzed at 0, 30, 60 and 90 days time interval for drug content, mean globule size, Polydispersiblity index, Zeta potential, % Transmittance, Self emulsification and Precipitation assessment test and *in vitro* dissolution profile.

Results and Discussion

Solubility study

The self-emulsifying formulations consisted of oil, surfactants, cosurfactants and drug should be a clear and monophasic liquid at ambient temperature when introduced to aqueous phase and should have good solvent properties to allow presentation of the drug in solution (Kommuru et al. 2001). The solubility of Efavirenz in various vehicles is presented in Table 3.

Table 3. Solubility study of efavirenz in various oil, surfactant and cosurfactant (n =3 ± sd).

Oil	Solubility ± S.D (mg/gm)	Surfactant and cosurfactant	Solubility ± S.D (mg/gm)
Labrafil M 2125 CS	135 ± 1.25	Polyethylene glycol 400	418 ± 1.25
Lauroglycol 90	76 ± 2.35	Tween 20	121 ±2.50
Caprol 90	52 ± 1.45	Tween 80	147 ± 3.45
Labrafac Lipophile WL 1349	155 ± 3.26	Propylene glycol	367 ±0.82
Labrafil M 2130 CS	115 ± 1.54	Cremophor EL	132 ± 1.23
Labrafac PG	122 ± 0.84	Gelucire 44/14	127 ± 2.56
Capmul MCM C8	79 ± 4.16	Labrasol	128 ± 3.43
Capmul MCM C10	84 ± 1.80	Trascutol HP	120 ± 4.45
Captex 200	96 ± 2.50	Caprol PGE 860	110 ± 1.85
Soyabean Oil	85 ± 0.50	Glycerol	74 ± 1.50
Safflower Oil	80 ± 0.75	Triacetin	123 ± 1.60
		Water	0.009 ± 2.25

As shown in Table 3, Labrafac Lipophile WL 1349, Labrafil M 2125 CS showed the highest solubilization capacity for Efavirenz among oils followed by Tween 80, Cremophore EL, Gelucire 44/14, PEG 400, Propylene glycol and Labrasol showed the highest solubilization capacity for Efavirenz among surfactants and co-surfactants. Thus, for further study we selected Labrafac Lipophile WL 1349 and Labrafil M 2125 CS as oils, Tween 80, Cremophore EL and Gelucire 44/14 as Surfactants, and PEG 400 and Labrasol as Co-surfactants.

Emulsification Studies

Labrafac Lipophile WL 1349 and Labrafil M 2125 selected as oils. Tween 80, Cremophore EL, Gelucire 44/14 and Tween 20 were selected as surfactants and PEG 400, Propylene Glycol, Labrasol, Transcutol HP and Triacetin were selected as cosurfactants for Emulsification Studies. Table 4 shows relative efficacy of surfactant and cosurfactants combination to emulsification of oil phase. When Tween 80, Cremophore EL and Gelucire 44/14 combined with PEG 400 and Labrasol appeared to be good emulsifiers for Labrafac Lipophile WL 1349 compare to Labrafil M 2125 may be due to Labrafac Lipophile have shorter alkyl chain length compare to Labrafil M 2125. Transcutol HP is Diethylene glycol monoethyl ether derivative and Triacetin have three alkyl chains were less effective as they could not improve emulsification of surfactants When Co-surfactants combines with Tween 20 appeared to be poor emulsifiers for both the Oils. As ratio of surfactant to cosurfactant is constant, study clearly distinguished ability of cosurfactants to improve emulsification of surfactants. Furthermore, as cosurfactants improve emulsification of surfactants by penetrating interfacial surfactant monolayer, their performance is affected by their structure and chain length (Malcolmson et al. 1998, Warisnoicharoen et al. 2000). Furthermore, Due to high solubilization potentials of Labrafac Lipophile, Tween 80, Cremophore EL, Gelucire 44/14, PEG 400 and Labrasol were used for further study.

The results for emulsification study are shown in Table 4.

Table 4. Emulsification studies on surfactant co-surfactant combination

Surfactant + Cosurfactant	Oil phase	
	Labrafac Lipophile WL 1349	Labrafil M 2125
Tween 80 + Polyethylene glycol 400	I	I
Tween 80 + Popylene glycol	I	I
Tween 80 + Labrasol	I	II
Tween 80 + Trascutol HP	II	II
Tween 80 + Triacetin	V	V
Cremophor EL + Polyethylene glycol 400	I	I
Cremophor EL + Popylene glycol	I	I
Cremophor EL + Labrasol	I	II
Cremophor EL + Trascutol HP	II	II
Cremophor EL + Triacetin	V	IV
Gelucire 44/14 + Polyethylene glycol 400	I	III
Gelucire 44/14 + Popylene glycol	II	II
Gelucire 44/14 + Labrasol	I	IV
Gelucire 44/14 + Trascutol HP	IV	IV
Gelucire 44/14 + Triacetin	V	V
Tween 20 + Polyethylene glycol 400	II	II
Tween 20 + Popylene glycol	III	III
Tween 20 + Labrasol	II	II
Tween 20 + Trascutol HP	III	II
Tween 20 + Triacetin	V	V

Pseudoternary Phase Diagrams

Self-microemulsifying systems form fine oil-water emulsions with only gentle agitation, upon their introduction into aqueous media. Surfactant and cosurfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence. The decrease in the free energy required for the emulsion formation consequently improves the thermodynamic stability of the microemulsion formulation (Patel et al. 2007). Therefore, the selection of oil and surfactant, and the mixing ratio of oil to S/CoS, play an important role in the formation of the microemulsion. The proper ratio of one excipient to another in the SMEDDS formulation was analysed. Several formulations with different surfactant and cosurfactant and S/ScoS values (the ratio of surfactant to cosurfactant) were dispersed with water. Figure 1 shows pseudo-ternary phase diagrams of the formulation composed of Labrafac Lipophile-Tween 80-PEG 400 and Figure 2 shows pseudo-ternary phase diagrams of the formulation composed of Labrafac lipophile-Tween 80-Labrasol with different S/CoS value. The size of the microemulsion region in the diagrams was compared, the larger the size the greater the self-microemulsification efficiency. Microemulsion formation area decreased with increased cosurfactant concentration and gave larger microemulsion region at surfactant to cosurfactant ratio (S/ScoS) = 1:1. Formulation was optimized using S/ScoS ratio 1:1.

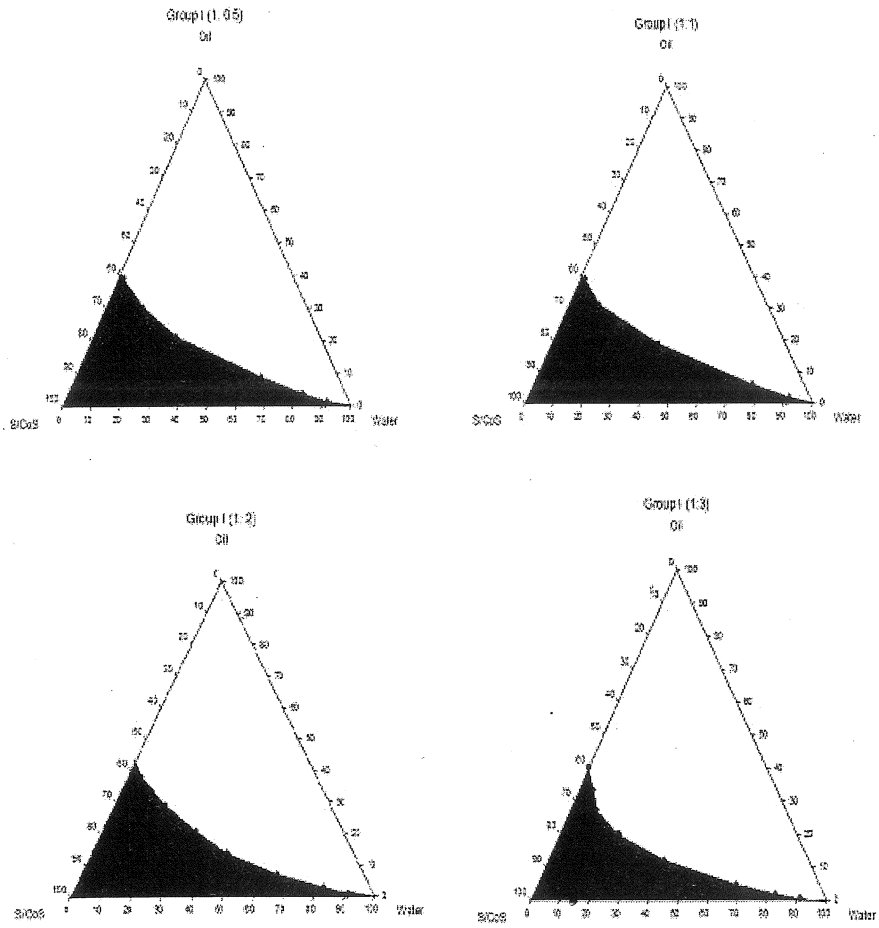

Figure 1. Pseudo ternary phase diagram of Labrafac lipophile, Tween 80 and Labrasol (Group I).

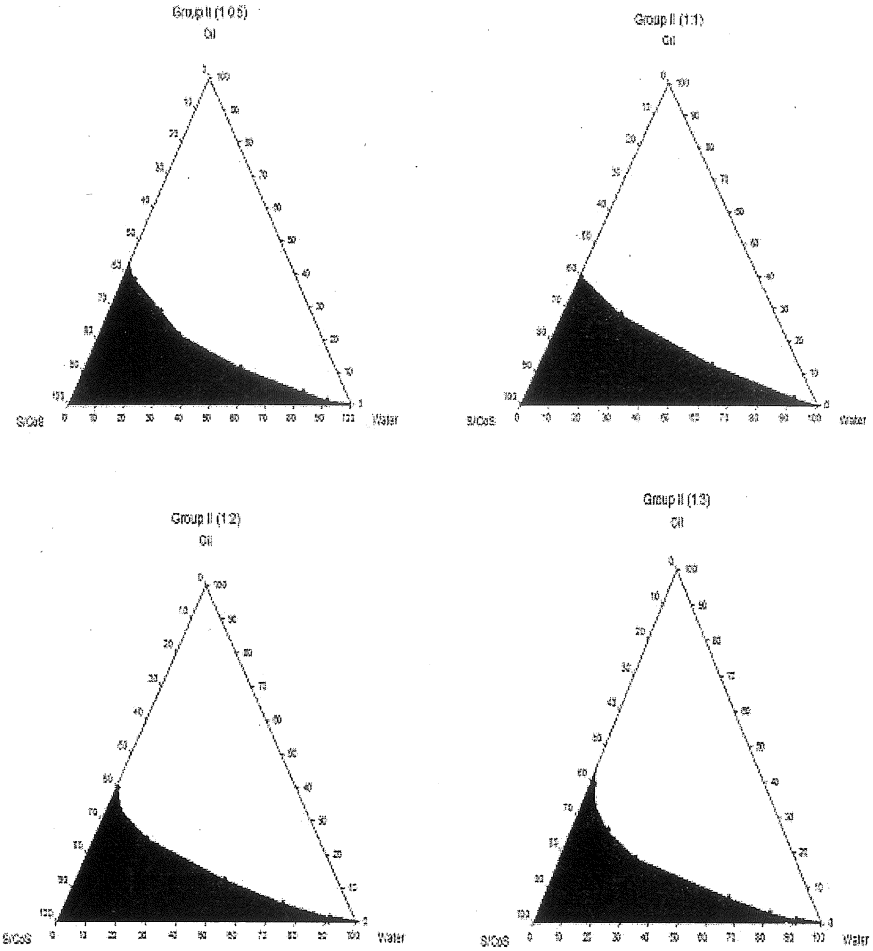

Figure 2. Pseudo ternary phase diagram of Labrafac lipophile, Tween 80 and PEG 400 (Group II)

Self emulsification and Precipitation Assessment test

The results of self emulsification and precipitation studies are given in Table 5. It was observed that formulation EF1 and EF4 come under categories of II as per grading system may be due to high concentration of Labrafac Lipophile and decreased concentration of surfactant and cosurfactant proportionally in formulations (Patel et al. 2007).

Freeze Thaw studies

The results of the Freeze thaw studies are given in Table 5. It was noticed that formulations EF1 and EF4 were unstable during freeze thaw cycle. It might be due to less concentration of surfactant and cosurfactant attribute to stabilized higher amount of Labrafac Lipophile in formulations.

Viscosity

Viscosity studies are necessary for SMEDDS to characterize the system physically and to control its physical stability. Viscosity is an important parameter for filling formulations in

hard gelatin capsules or soft gelatin capsules for commercialization. The viscosity of the formulations was determined and the values are shown in Table 5.

Table 5. Observation of self emulsification and precipitation, freeze thaw study and viscosity of various formulations

SMEDDS Formulations	Self emulsification and Precipitation Assessment test				Freeze thaw study	Viscosity In Cps ± S.D.
	In Distill water		In 0.1 N HCl			
	Initial	After 48 h	Initial	After 48 h		
EF1	I	II	I	II	Unstable	275 ± 6.84
EF2	I	I	I	I	Stable	287.5 ±5.59
EF3	I	I	I	I	Stable	300 ± 6.84
EF4	I	II	I	II	Unstable	312.5 ±5.59
EF5	I	I	I	I	Stable	325 ± 5.59
EF6	I	I	I	I	Stable	337.5 ± 6.84

Percentage transmittance

The percentage transmittance of the SMEDDS formulations after 50 times and 100 times dilution with 0.1 N HCl and distilled water are shown in Table 6. All the formulations showed closer to 100% transmittance indicated clear and transparent appearance.

Table 6. Percent transmittance of various formulations at various dilutions in water and 0.1N HCl.

SMEDDS formulations	% Transmittance ± S.D.			
	50 times dilution with distill water	100 times dilution with distill water	50 times dilution with 0.1 N HCl	100 times dilution with 0.1 N HCl
EF1	95.56 ± 0.025	98.12 ±0.052	95.13 ± 0.082	98.15 ± 0.062
EF2	99.30 ± 0.012	99.78 ± 0.020	99.24 ± 0.024	99.72 ± 0.053
EF3	99.12 ± 0.032	99.52 ±0.017	98.85 ± 0.036	99.42 ± 0.031
EF4	96.32 ± 0.016	98.23 ± 0.024	95.76 ± 0.024	98.12 ± 0.023
EF5	99.23 ± 0.021	99.82 ± 0.030	99.12 ± 0.036	99.78 ± 0.012
EF6	99.08 ± 0.031	99.52 ± 0.059	99.05 ± 0.026	99.63 ± 0.027

Droplet size/Polydispersibility index (PDI) and Zeta potential

The effect of the formulation of SMEDDS on the droplet size, polydispersibility index (PDI) and Zeta potential in distill water and 0.1 N HCl is shown in Table 7.

Table 7. Droplet size, polydispersibility index and zeta potential of SMEDDS formulations

SMEDDS Formulations		EF1	EF2	EF3	EF4	EF5	EF6
In Distill water	Droplet size	113	91.2	96.7	80.5	58.9	63.4
	PDI	0.25	0.12	0.26	0.23	0.18	0.26
	Zeta potential	-15.7	-17.7	-17.5	-14	-16	-16.2
In 0.1 N HCl	Droplet size	123	93.9	105	90.6	62.8	70.2
	PDI	0.27	0.14	0.29	0.25	0.22	0.29
	Zeta potential	-16.6	-18.8	-18.4	-16	-18.2	-18.6

Droplet size is important parameter for dissolution enhancement and hence bioavailability. There were minor differences in mean droplet size observed between diluting with distill water and with 0.1M HCl. The droplet size of the SMEDDS formulation containing PEG 400 as a co surfactant was smaller than that of the SMEDDS formulation containing Labrasol as a

co-surfactant. Labrasol containing formulation EF2 showed average droplet size 91.2 nm (Figure 3a) and 93.9 nm (Figure 3b) in distilled water and 0.1N HCl respectively while PEG 400 containing formulation EF5 showed average droplet size 58.9 nm (Figure 3c) and 62.8 nm (Figure 3d) in distilled water and 0.1N HCl respectively and have selected for further study.

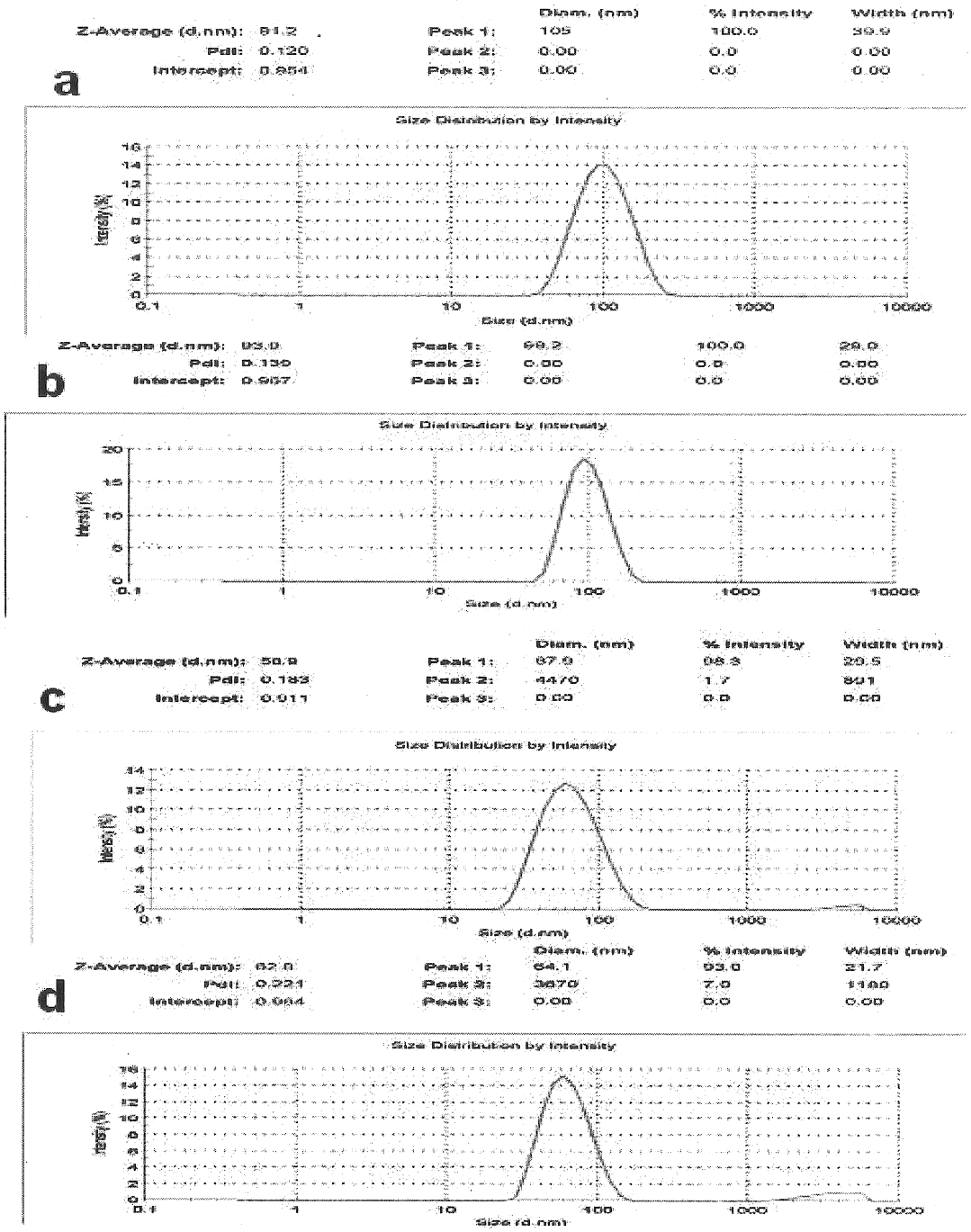

Figure 3. Globule size of formulation EF2 in distilled water (a) and 0.1N HCl (b); formulation EF5 in distill water (c) and 0.1N HCl (d).

Polydispersity index (PDI) is a measure of particle homogeneity and it varies from 0.0 to 1.0. All the formulations showed less than 0.26 and 0.29 in distilled water and 0.1N HCl respectively. The closer to zero the polydispersity value the more homogenous are the particles (Lawrence et al. 2000). So from the results it can be concluded that all the formulations were homogenous.

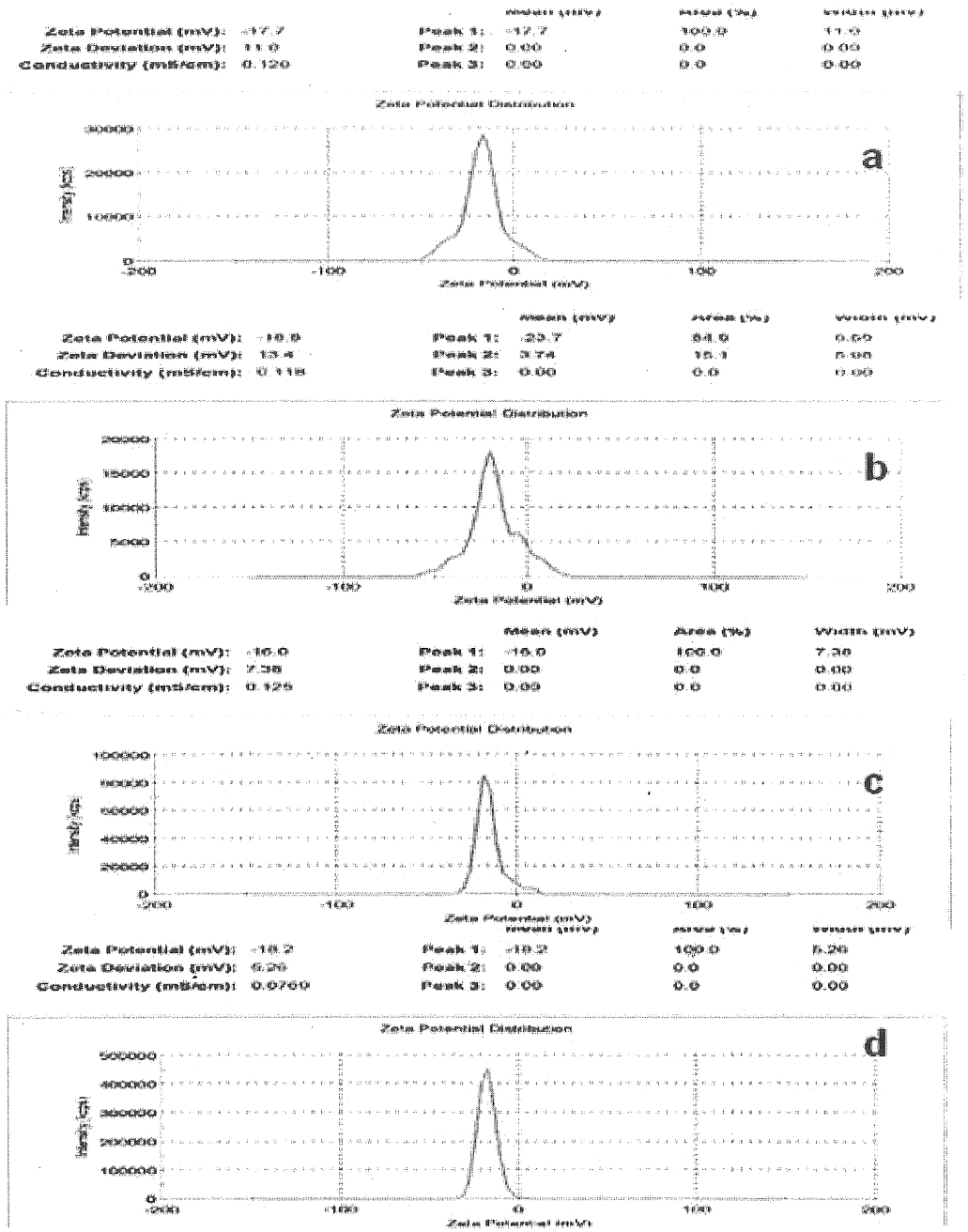

Figure 4. Zeta potential of formulation EF2 in distilled water (a) and 0.1N HCl (b); formulation EF5 in distill water (c) and 0.1N HCl (d).

The significance of zeta potential is that it can be related to the stability of colloidal dispersions. Zeta potential indicates degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules that are small enough a high zeta potential will confer stability, i.e., the solution or dispersion will resist aggregation. Zeta potential controls charge interactions. Conventionally, a high zeta potential can be high in positive or negative sense, i.e., −30mV or +30mV would be considered as high zeta potential. Formulation EF2 showed Zeta potential of -17.7 mV (Figure 4a) and -18.8 mV (Figure 4b) in distilled water and 0.1N HCl respectively while formulation EF5 showed Zeta potential of -16.0 mV (Figure 4c) and -18.2 mV (Figure 4d) in distilled water and 0.1N HCl respectively. Negative values of zeta potential of the optimized formulations indicated that the formulations were negatively charged.

Transmission Electron Microscopy (TEM)

The morphology of SMEDDS was examined with a transmission electron microscope. The droplet on the microemulsion appears dark with the bright surroundings. TEM photographs (Figure 5) further conformed that the globules are spherical in shape.

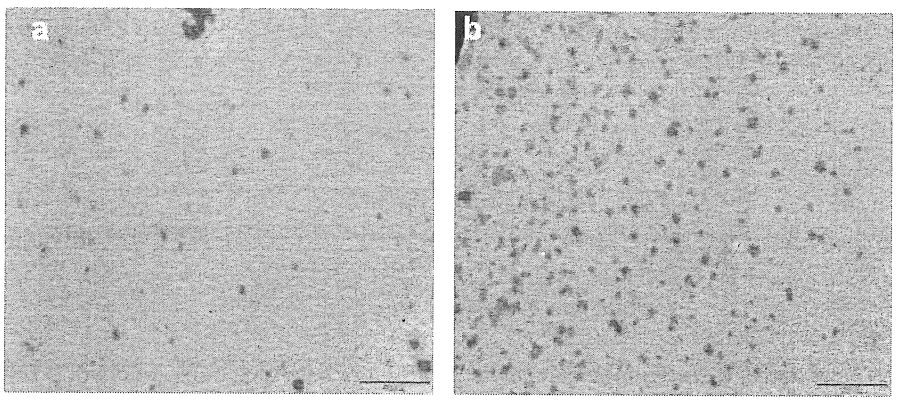

Figure 5. TEM images of formulation EF2 (a) and EF5 (b).

In Vitro Dissolution Studies

Based on the aforementioned study, dissolution study of capsules filled with two optimal SMEDDS (i.e. formulations EF2 and EF5), plain Efavirenz and the marketed formulation (Efavir, Cipla ltd) was performed. The release profile of Efavirenz was investigated in water containing 1.0% SLS (Figure 6a), 0.1N HCl (pH 1.2) containing 1.0 % SLS (Figure 6b) and acetate buffer (pH 4.5) containing 1.0 % SLS (Figure 6c). Both the SMEDDS formulations released above 90% Efavirenz in 15 min in all three dissolution media. Market sample released around 30 to 40 % efavirenz in 15 min and plain Efavirenz released around 13 to 20 % after 15 min in all three dissolution media. The release of drug from the SMEDDS formulations was highly significant ($p<0.05$) when compared with marketed formulation and plain drug. Dramatic increase in the rate of release of Efavirenz from SMEDDS formulation compared to marketed formulation and plain drug can be attributed to its quick dispersability, ability to keep drug in solubilized state and small droplet size provided a large surface area for the release of the drug and thus permitting faster rate of drug release. It was also evident that release of Efavirenz from SMEDDS was independent of pH dissolution medium.

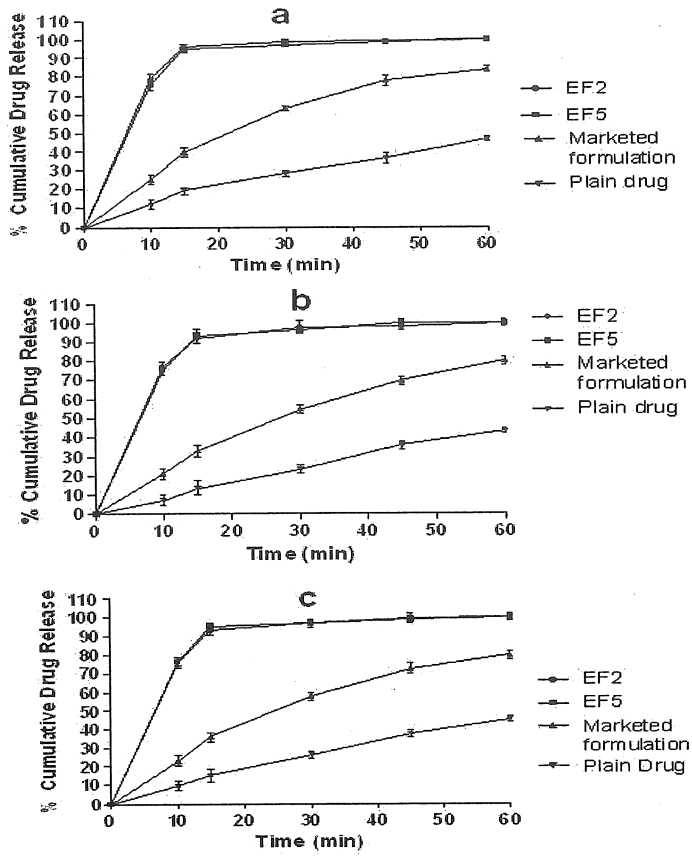

Figure 6. Comparative dissolution profile in distilled water containing 1.0 % SLS (a), 0.1N HCl (pH 1.2) containing 1.0 % SLS (b) and acetate buffer (pH 4.5) containing 1.0 % SLS (c) of various formulations (n=3 ± SD).

Pharmacokinetic study in rats

The Marketed formulation (Efavir, Cipla ltd) and the capsules filled with the two SMEDDS formulations (i.e. formulations EF2 and EF5) were used for the bioavailability study. Pharmacokinetic parameters and the relative bioavailability of Efavirenz after oral administration of the three formulations to albino rats are shown in Table 8.

Table 8. Relative bioavailability and pharmacokinetic parameters of efavirenz from orally administered different formulations to male albino rats.

Formulations	**EF2**	**EF5**	**Marketed formulation**
Tmax in hrs	3	3	4
Cmax (µm/ml)	8.72	9.12	5.48
($AUC_{0\rightarrow 48}$) (µg·h/ml)	234.6	238	131.78
($AUC_{0\rightarrow \infty}$) (µg·h/ml)	318.9	328.1	162.89
Relative bioavalability (%)	178	180.6	100

The plasma profiles of Efavirenz in rats following oral administration of the different formulations are represented in Figure 7. The Cmax and $AUC_{0\rightarrow48}$ h of the SMEDDS were significantly higher than those of the marketed formulation. The Tmax of the SMEDDS was less than that of the marketed formulation. The relative bioavailability of SMEDDS form to the marketed formulation was calculated using the following equation:

Relative BA (%) = [AUC test / AUC Reference] x 100

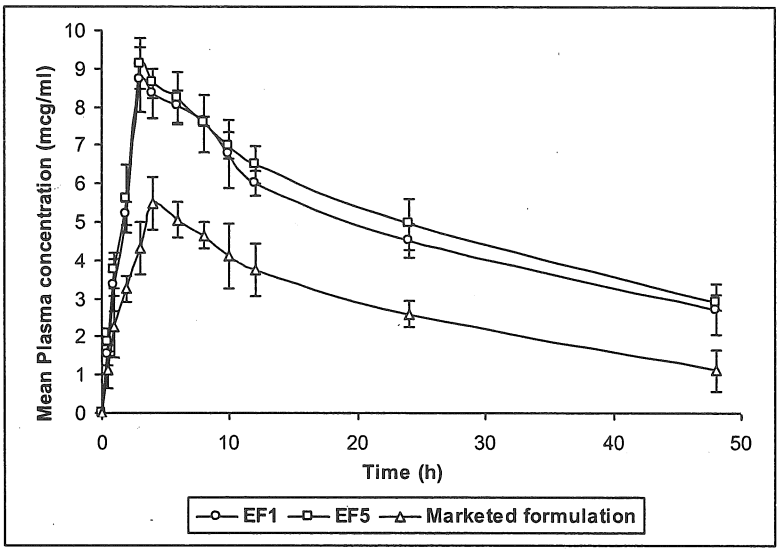

Figure 7. Mean plasma concentration (mcg/ml, n=3 ± SD) in male Albino rats after oral administration of EF1, EF5 and marketed formulations.

The relative bioavailability of Efavirenz in formulations EF2 and EF5 was about 1.77 and 1.80 fold higher compared with the marketed formulation. The difference between the SMEDDS formulations and marketed formulation is statistically significant ($p<0.05$). This might be the solubilization and droplet-size reduction produced by SMEDDS. SMEDDS could increase the oral bioavailability of Efavirenz. It might be a promising approach for rapid onset and effective absorption with oral administration of Efavirenz.

Stability Studies

No change in the physical parameters such as homogeneity and clarity was observed during the stability studies. Interestingly, no significant decline in the Efavirez content was observed at the end of 90 days at accelerated condition (40°C/75% RH) indicating that Efavirenz remained chemically stable in SMEDDS formulation. It was also seen that the formulation EF2 was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation observed. Furthermore, no significant change in other parameters such as *in vitro*, dissolution profile, globule size, Zeta potential, %Transmittance and self-microemulsion efficiency was observed for the Efavirenz SMEDDS formulations. Thus, these studies confirmed the stability of the developed SMEDDS formulation.

Conclusion

SMEDDS formulations consist of lipids, surfactants and cosurfactants which are emulsified by aqueous medium under gentle digestive motility in the gastrointestinal tract. It is considered that the excipients in SMEDDS could increase the dissolution and permeability of drug by significantly decreasing droplet size and restraining the secretion of drug efflux transporter P-gp. Efavirenz is a representative poorly water-soluble drug that is used as anti HIV drugs. In the present study various SMEDDS formulations containing efavirenz were prepared and studied for their various characteristics. The concentration of Efavirenz in various excipients was analysed. Pseudo-ternary phase diagrams composed of lipid–cosurfactant–surfactant–water were mapped; the microemulsion region in each diagram was plotted and compared. Droplet size and distribution, zeta-potential and long-term stability were investigated in detail. Results from the stability studies at 40±2 °C and 75±5% RH indicated stability of the optimized formulation as there was no significant change in the observed physical and chemical parameters. Optimal formulations that contained Tween 80 as surfactant, PEG 400 or Labrasol as cosurfactant and Labrafac lipophile as lipid can become microemulsions when dispersed with aqueous medium. The average droplet size of the optimal SMEDDS formulation is within 100 nm. The rate and amount of the release of Efavirenz from SMEDDS capsules were more than those from the marketed formulations and plain drug in distilled water, 0.1N HCl (pH 1.2) and acetate buffer (pH 4.5). Efavirenz SMEDDS formulations were assessed for its *in vitro* dissolution and an oral bioavailability in albino rats. Efavirenz SMEDDS formulations were found to be superior to commercial formulation with respect to *in vitro* dissolution profile and *in vivo* bioavailability and they might have the potential to advance the oral bioavailability of efavirenz. After oral administration of efavirenz to rats, the relative bioavailability of SMEDDS formulation EF2 and EF5 to the marketed formulation (Efavir, Cipla ltd) was 177 % and 180%, respectively. Our study indicates that the potential use of SMEDDS for the oral delivery of efavirenz can be an alternative to improve its systemic availability. The development of SMEDDS is promising for improving the oral bioavailability of poorly soluble drugs. In the future, a human study after an oral administration of efavirenz is also required for its clinical use.

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