Chapter 2

Preparation and characterization of nevirapine self-emulsifying formulation containing Soluphor® P

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Abstract

Nevirapine (NVP) is a highly lipophilic and water insoluble non-nucleoside reverse transcriptase inhibitor (NNRTI) used for the treatment of HIV-1 infection. Lymphoid tissue constitutes the major reservoir of HIV virus and infected cells in HIV-infected patients. Self-emulsifying drug delivery system (SEDDS), using long chain triglycerides (LCT), is a popular carrier of drugs due to their ability to transport lipophilic drugs into the lymphatic circulation. However, HIV/AIDS patients experience a variety of functional and anatomical abnormalities in gastrointestinal tract that result in diarrhoea and nutrient malabsorption. Fat malabsorption leads to steatorrhea, weight loss, general debilitation and malnutrition. Medium chain triglycerides (MCT) are readily absorbed from the small bowel under conditions in which the absorption of LCT is impaired. Therefore, NVP SEDDS containing medium chain fatty acid, caprylic acid and a solubilizer, Soluphor® P (2-pyrrolidone) was developed and found to be superior to the marketed conventional suspension with respect to in vitro diffusion and ex vivo intestinal permeability. This SEDDS was further investigated for in vivo absorption in an animal model. The contribution of caprylic acid and Soluphor® P on in vivo absorption of NVP was also studied in the present study. The bioavailability of NVP from SEDDS, after oral administration, was 2.69 times higher than that of the marketed suspension. The present investigation indicates that, Soluphor® P has the potential to enhance the in vivo absorption of poorly water soluble drug when used as a solubilizer in a self emulsifying drug delivery system. The improved bioavailability could be due to absorption of NVP via both portal and intestinal lymphatic routes. The study indicates that medium chain or structured triglycerides can be a better option to develop SEDDS for lipophilic and extensively metabolised drugs like nevirapine for patients with AIDS-associated malabsorption.
2.1. Introduction

The pharmacological activity is often accompanied by problematic physicochemical and biological properties such as poor solubility and permeability. All too often, the poor bioavailability resulting from solubility and/or permeability problems are identified at a rather late stage of the development process, making it difficult to modify the chemical structure of the compound and forcing pharmaceutical companies to invest resources in formulation development of new chemical entities (NCEs) to a virtually unprecedented extent.

Antiretroviral therapy for the treatment of human immunodeficiency virus type 1 (HIV-1) infection has improved steadily since the advent of potent combination therapy in 1996. New drugs have been approved that offer new mechanism of action, improvements in potency and activity even against multi-drug–resistant viruses, dosing convenience, and tolerability. Despite the wide variety of highly potent anti-HIV or anti-retroviral (ARV) drugs that have been developed and made available in clinical practice over the years, total eradication of HIV infection has not been achieved. Currently, HIV infection and AIDS are thought to be only controlled. HIV attacks host immune cells namely macrophages and CD4+T-cells and sequesters itself into sanctuary and reservoir sites such as the lymphoid tissues, testes, and brain (Pomerantz, 2003; Saksena et al., 2010). Strategies currently being investigated to overcome these limitations include the identification of new and chemical modification of existing chemical entities, the examination of various dosing regimens, as well as the design and development of novel drug delivery systems (NDDS) that can improve the efficacy of both existing and new ARV drugs. More specifically, in the past decade there has been an explosion of interest in the development of NDDS for the incorporation of ARV drugs as a way of circumventing the problems described above and optimising the treatment of HIV/AIDS patients. There has been significant advancement of the systems as described by Mirchandani & Chien (1993), and further new NDDS for ARV drugs have since emerged in the literature. The drug delivery efforts should focus on improving the physicochemical (i.e. solubility), biopharmaceutic (i.e. absorption, metabolism), and pharmacokinetic (i.e. blood concentrations) properties of the parent drugs. To effectively control HIV infection, however, will require advanced drug delivery approaches in order to access and maintain effective drug concentrations for prolonged periods of time in sanctuary sites.
A hypochlorhydria (reduced gastric acid secretion) is reported to be common in patients with AIDS (McConnell et al., 2008; Wilcox et al, 1999). Such condition hampers the oral absorption of various weakly basic drugs like nevirapine, itraconazole, ketoconazole, etc.

Nevirapine, the most important drug used in the highly active antiretroviral therapy (HAART), is a BCS class II compound with poor aqueous solubility and optimum permeability, and poses a challenge in achievement of optimal dissolution kinetics from the dosage forms. The pH solubility profile indicates a gradual decline in solubility with an increase in pH from 1.5 (1.9 mg/mL to 4 (0.1 mg/mL) and remained steady at higher pH (0.1 mg/mL at pH 8) (Macha et al., 2009). Nevirapine, particularly at higher dose (> 50 mg) exhibits characteristics of solubility rate limited absorption with a resultant decrease in bioavailability (Sarkar et al., 2008).

The use of lipid and surfactant based formulations is one of the several approaches that has been applied in order to improve the oral bioavailability of poorly aqueous soluble compounds intended for oral administration. The approach has proved efficient and hence has received much attention. Lipid and surfactant based systems include systems like emulsions, microemulsion, self-emulsifying drug delivery systems (SEDDS), micellar solutions, and dry emulsions like lipid solutions and lipid suspensions. The number of excipients which are pharmaceutically acceptable and applicable in the formulation of lipid and surfactant based systems is large. The use of solubilising excipients in marketed formulations of poorly soluble compounds has been reviewed recently (Strickley 2004).

The oil component of SEDDS is an important constituent, not only because it can solubilise lipophilic drug or facilitate self emulsification but it can also increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the gastrointestinal tract depending on the nature of the triglyceride (Charman and Stella, 1991; Gershanik and Benita, 2000; Holm et al., 2002).

Excipients employed for such delivery systems are susceptible to enzymatic degradation in the GI tract. Excipients susceptible to degradation include natural di- and triglycerides, as well, as some commonly used surfactants like Labrasol, Labrafils, Gelucires, Cremophors, Tween 80 (Khosravi et al 2002, Seebaluck et al 2003).

Recently, it has been demonstrated that some of the excipients used as surfactant should be considered as more than just inert substances. It has been shown that Cremophor EL, Tween 80, Labrasol, Miglyol polyethoxylated, can inhibit the PGP efflux transporter (p-
glycoprotein) (Cornaire et al 2004; Hugger et al 2002; Shono et al 2004;) and hereby potentially improve bioavailability of drug molecules being PGP substrates. A number of excipients have been shown to influence the lymphatic transport both in rats, and have an impact to the chylomicron secretion in caco-2 cells, which is also believed to be an indicator of lymphatic transport (Karpf et al 2004; Rege et al 2002; Seeballuck et al 2003).

2-Pyrrolidone (2P) is an organic, colorless, readily biodegradable, water-miscible liquid with solubilizing properties. It has a partition coefficient \( \log K_{ow} \) of \(-0.71\), a melting point of 25.5 °C and a boiling point of 245 °C. 2-Pyrrolidone lacks mutagenic or genotoxic activity. It has a high oral LD\(_{50}\) of 5000 mg/kg body weight in rats, a low aquatic hazard, a low developmental toxicity and no reproductive toxicity (EPA website: http://www.epa.gov/oppt/chemrtk/ pubs/summaries/2pyrroli/c14223rt.pdf). Although 2-pyrrolidone has a potentially hydrolysable amide group, amides are considered resistant to hydrolysis in a pH range of 1.0–8.0, and require prolonged heating in strong base or acid to accomplish hydrolysis (Volhardt, 1987). These observations suggest that 2-pyrrolidone could be used safely as a solubilizer or a solvent, environmentally as well as pharmacuetically.

2-pyrrolidone is a normal component of the diet in humans, is of endogenous origin, rapidly metabolised after oral and parenteral administration in mammalian species. 2-pyrrolidone is able to cross the blood-brain barrier and has been shown to be hydrolysed enzymatically to gamma amino butyric acid \textit{in vivo} (EMEA/MRL/457/98 http://www.eudra.org/emea.html).

We have made an attempt to develop a self-emulsifying drug delivery system of nevirapine using 2-pyrrolidone (Soluphor\textreg; P) as a solubilizer with a view to improve solubility and \textit{in vivo} absorption via intestinal lymphatics in a rat model. Soluphor P has not been explored fully as a solubilizer.

The aim of the study was to determine the solubilising efficiency of Soluphor\textreg; P (2-pyrrolidone) and to explore its potential for developing a SEDDS for model investigational lipophilic BCS Class II drug, NVP. The developed formulation was characterised for droplet size after dilution in aqueous media, \textit{in vitro} diffusion studies, \textit{ex vivo} permeation across rat stomach and intestine and \textit{in vivo} absorption in rat.
2.2 Materials and methods

2.2.1. Materials

Nevirapine was a generous gift from Matrix Laboratories Ltd. (Hyderabad, India). Caprylic and Oleic acids were obtained from Soofi Traders (Mumbai, India); Polyoxyl 35 castor oil (Cremophor EL; HLB 12-14) and 2-Pyrrolidone (Soluphor® P) were obtained from BASF Corp. (Mumbai, India). Diethylene glycol monoethyl ether (Transcutol P®; HLB 4.2), Propylene glycol monocaprylate (Capryol 90® HLB 6) were provided by Gattefosse, France and Glycerol Monocaprylocaprate (Capmul MCM® HLB 5-6) was provided by Abitec Corp., Janesville WI. Tween 20 (HLB 17), Tween 80 (HLB 15) and diethyl ether were purchased from S D Fine chemicals (Mumbai, India). Carbamazepine was purchased from HIMEDIA. All other chemicals and solvents used were of analytical grade.

2.2.2. Solubility studies

The solubility of nevirapine in various oils, surfactants and co-surfactants was studied. An excess of nevirapine was placed in 2 mL of various vehicles in a screw-capped glass vial and the mixture was heated at 60°C in a water-bath (Equitron, Chennai, India) to facilitate the solubilisation using a cyclomixer (CM 101, Remi Instruments Ltd, Ahmedabad, India). Mixtures were equilibrated at 30°C for 48 h in a water bath and then centrifuged in a laboratory centrifuge at 2000 rpm for 15 min to separate the undissolved drug. Aliquots of supernatant were diluted with methanol and analysed for the dissolved drug by HPLC (Jasco, Japan), using RP column (LCGC Qualisil BDS C18; 5 µm 250 mm x 4.6 mm i.d) and methanol: water (50:50) as a mobile phase (Anbazhagan et al., 2005).

2.2.3. 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 (Djordjevic et al., 2004). Ternary plots were constructed using Caprylic acid, Soluphor P and Transcutol P as oil, surfactant and co-surfactant respectively, in different ratios of Soluphor P : Transcutol P (1:4, 1:3, 1:2 1:1 and 4:1 w/w) with Caprylic acid in ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 w/w.

The mixtures of oil and 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 subjected
2.2.4. Preparation of nevirapine self emulsifying drug delivery system

SEEDS formulation was developed based on the microemulsion regions and maximum amount of drug that can be solubilised in a particular ratio of surfactant and co-surfactant with Caprylic acid meeting the desired criteria for formation of microemulsion after dispersing in aqueous media. The developed formulation consisted of nevirapine: caprylic acid: Soluphor P: Transcutol P (4.5: 8.17: 71.16: 16.17) (% w/w). The formulation was prepared by dissolving the nevirapine in the mixture of Transcutol P and Soluphor P at 50°C in a water bath. Caprylic acid was then added. This mixture was mixed by vortexing using cyclomixer until a transparent preparation was obtained. The prepared nevirapine SEDDS can be delivered in hard gelatin capsule.

2.2.5. Characterization of self emulsifying drug delivery system

2.2.5.1. Drug content

SEDDS formulation equivalent to 50 mg of NVP was taken and diluted in methanol. Volume was made up to 50 mL with methanol (1mg/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 (Shimadzu UV-2450, Japan) (Anbazhagan et al., 2005; Sarkar et al., 2006). Placebo sample was also treated in the same way to check the interference, if any. Methanol was used as a reference solution.

2.2.5.2. Spectroscopic characterization of optical clarity

The optical clarity of aqueous dispersions of SEDDS formulation was measured spectrophotometrically. Composition was prepared according to the design and diluted to 100 times with distilled water, 0.1N HCl and phosphate buffer pH 6.8. The % transmittance of solution was measured at 650 nm, using distilled water as a reference (Cirri et al., 2007; Subramanian et al., 2004; Zhang et al., 2008).

2.2.5.3. Morphological characterization

The morphology of self emulsifying formulation was observed by using a transmission electron microscope (TEM) (Phillips Tecnai 20, Holland) at an acceleration voltage of 200
kV and typically viewed at a magnification of 43,000x. The size of the colloidal structures was determined using AnalySIS® software (Soft Imaging Systems, Reutlingen, Germany).

Formulation was diluted with distilled water 1:25 and shaken. Carbon-coated copper grids were glow-discharged (Edwards E306A Vacuum Coater, England) and 10 µL of sample adsorbed on to these holey film grid and observed after drying.

2.2.5.4. Determination of droplet size and zeta potential

Droplet size and the zeta potential of the formed emulsion were determined by photon correlation spectroscopy (PCS) that analyzes the fluctuations in light scattering due to Brownian motion of the particles, using a Zetasizer ZS 90 (Malvern Instruments, UK). Light scattering was monitored at 25°C at a 90° angle.

2.2.5.5. In vitro diffusion study

*In vitro* diffusion study of the nevirapine SEDDS was compared with a conventional marketed suspension (Nevimune®, Nevirapine Oral Suspension, CIPLA) using a dialysis technique (Dixit et al., 2010; Kang et al., 2004; Kim et al., 2000; Patil et al., 2004). The dialyzing medium was 0.1N HCl. 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, equivalent to 100 mg drug, 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 100 rpm over a magnetic stirrer (Remi Instrument Ltd., Mumbai, India) at 37°C. Aliquots of 1 mL were removed at 15, 30, 45, 60, 120, 180, 240 and 300 min time intervals and suitably diluted further. Each time the volume of aliquots was replaced with the fresh dialyzing medium. These samples were analyzed for NVP present in the dialyzing medium at corresponding time by UV-visible spectrophotometer (Shimadzu UV-2450, Japan) at 313 nm.

2.2.5.6. Ex vivo stomach permeability studies

Male Sprague-Dawley rats (250-300 g) were euthanized in carbon-dioxide vacuum chamber. All experiments and protocols described in this animal study were approved by the Institutional Animal Ethics Committee of B V Patel PERD Centre, Ahmedabad, India and were in accordance with the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. To check the stomach permeability, the stomach part was isolated carefully and taken for the *ex vivo* permeation study. Then this tissue was washed with
physiological acid solution containing 100 mM HCl and 54 mM NaCl to remove the mucous and gastric contents. The method employed was modified from experimental procedures well described in the literature (Gharzouli et al., 1995; Hung and Neu, 1997; Shah and Khan, 2004).

SEDDS formulation, 0.1 mL, (equivalent to 5 mg of drug) was injected into the stomach using a syringe, and the two sides of stomach tissue were tightly closed with the help of threads. In a similar way 0.5 mL (equivalent to 5 mg of drug) market suspension formulation (Nevimune®-CIPLA) was also filled in the stomach tissue. The filled stomach tissue was placed in a beaker with constant stirring and temperature of 37° C on a magnetic stirrer. The receiver compartment was filled with 30 mL of 100 mM HCl. Aliquots of 3 mL were withdrawn at 0.5, 1.0, 2.0 and 3.0 hours time intervals and suitably diluted further. The absorbance was measured using a UV-Visible spectrophotometer at a wavelength of 313 nm, keeping the respective blank. The percent permeation of drug was calculated against time and plotted on a graph.

2.2.5.7. *Ex-vivo* intestinal permeability studies

This was carried out by the method well described in the literature (Araya et al., 2006; Ghosh et al., 2006). Male Sprague- Dawley rats (250-300 g) were euthanized in carbon-dioxide vacuum chamber. All experiments and protocols described in this animal study were approved by the Institutional Animal Ethics Committee of B V Patel PERD Centre, Ahmedabad, India and were in accordance with the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. To check the intestinal permeability, small portion of small intestine was isolated and used for the *ex vivo* permeability study. The tissue was thoroughly washed with pH 6.8 phosphate-buffered saline (USP 30) (USP 30/NF25) to remove any mucous and lumen contents. The nevirapine SEDDS, 0.5g (equivalent to 25mg nevirapine) and conventional marketed suspension, 1ml (equivalent to 10 mg nevirapine) were diluted up to 10 mL and 4 mL with distilled water, respectively and mixed over cyclomixer for 1 minute. The resultant samples (2.5 mg/mL) of both nevirapine SEDDS and conventional marketed suspension were injected separately into the lumen of the small intestine tissue using a syringe, and the two sides of the intestine were tightly closed with the help of a thread. The tissue was placed in a beaker filled with 30 mL of pH 6.8 phosphate-buffered saline containing 20% PEG-400 with constant stirring at 37°C. The two ends of tissues were fixed horizontally on to a beaker with the
help of a thread. Aliquots of 3 mL were withdrawn at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4 and 5 hours time intervals and suitably diluted further. The absorbance was measured using a UV-Visible spectrophotometer at a wavelength of 283 nm (Kabra et al., 2009; Mandloi et al., 2009). The amount of drug diffused (%) was calculated against time and plotted on a graph.

2.2.6. HPLC method development and validation

The concentration of nevirapine in the plasma samples were analyzed by modified reported methods (Hollanders et al., 2000; Kabra et al., 2009). The HPLC apparatus consisted of Jasco PU-980 Intelligent HPLC pump (Jasco, Japan) equipped with a Jasco UV-975 Intelligent UV/VIS detector (Jasco, Japan), an autosampler Jasco AS-950-10 Intelligent sample (Jasco, Japan), a Jasco Borwin Chromatography Software (version 1.50) integrator software and a LCGC Qualisil BDS C18 (4.6 mm × 250 mm and 5 μm particle size) column. The mobile phase consisted of a mixture of 10 mM sodium phosphate buffer pH 5.0 and acetonitrile (70:30 v/v) at a flow rate of 1.0 mL/min that led to retention time of 6.75 min when detection was carried out at 245 nm. The assay was linear ($r^2=0.999$) in the concentration range of 25-5000 ng/mL. The method was validated with respect to accuracy and inter- and intra-day precision at three quality control samples (75, 500 and 3500 ng/mL) as per ICH guidelines (ICH Q2(B))

2.2.7. Bioavailability studies

Bioavailability studies were performed in male Wistar rats, weighing 280 to 350 g. All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee of Sri Dhanvantary Pharmaceutical Analysis and Research Centre, Surat, India and were in accordance with guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. Two groups were made for the study, and four rats were kept in each group. The animals were kept under standard laboratory conditions, temperature at 25 ± 2°C and relative humidity (55 ± 5%). The animals were housed in polypropylene cages, four per cage, with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water ad libitum. The animals were fasted overnight prior to the experiment but had free access to water. The formulations (SEDDS and marketed suspension (Nevimune®, B.No. G90908, MFD Sep 09, EXP AUG 12)) were given orally using feeding snode. Dose for the rats was selected as reported (Rao and Shinde, 2009)
and calculated based on the weight of the rats (20mg/kg body weight) according to the surface area ratio (Akhila et al., 2007; Paget and Barnes, 1964). The animals were anesthetized using ether and blood samples (approximately 500 μL) were collected from the retro-orbital vein using a heparinized needle (18-20 size) at 0 (pre-dose), 1, 2, 4, 8, 12, 16 and 24 hours after oral administration of formulations. The blood samples were collected into vacutainer tubes with flocculent anticoagulant powder (13 x 75mm, 2mL, Accuvac, BD, NJ), mixed and centrifuged on a laboratory centrifuge at 5000 rpm for 20 min at ambient temperature. The supernatant plasma was carefully separated and kept at –20°C (Thermo Scientific, USA) until analysis was carried out using validated HPLC.

2.2.7.1. Plasma analysis

Frozen plasma samples were thawed just prior to extraction. A 100 μL of plasma sample was transferred in 2 mL centrifuge tube (Tarson, Kolkata, India). To that 100 μL of Carbamazepine (1μg/mL in methanol) was added as an internal standard (Minizi and Ngaimisi, 2010) and vibrated on cyclomixer for 10 s. Then, 25μL of 0.1M sodium hydroxide was added, vortex-mixed for 20 s and to it 1mL of ethyl acetate was added and vortexed for 2 min. The tube was centrifuged at 15,000 rpm for 20 min at 4° C; the organic layer was collected (top layer) and was transferred to clean tube and dried under nitrogen stream at ambient temperature. The residue was reconstituted with a 100 μL aliquot of mobile phase, and 50 μL was injected directly onto the HPLC column at a flow rate of 1.0 mL/min.

Plasma concentration versus time data of nevirapine for rats was analyzed using standard non-compartment analysis. The area under the plasma concentration-time curve (AUC$_{0→t}$) from zero to 24 hour was estimated by the linear trapezoidal method (Lee and Ku, 1999; Han and Lee, 1999). The relative bioavailability ($F$) of SMEDDS to the suspensions was calculated using the following equation:

$$ F = \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{reference}}} \times 100\% $$

2.2.8. Statistical analysis

Statistical analysis for the determination of differences in diffusion, permeability and in vivo absorption profiles of nevirapine SEDDS and the marketed preparation was assessed by the use of Student’s t-test. The pharmacokinetic data between different formulations were compared for statistical significance by Student’s t-test. Statistical probability ($P$) values less than 0.05 were considered significantly different.
2.2.9. Stability studies:

Chemical and physical stability of nevirapine SEDDS (S1) were assessed under various storage conditions namely room temperature (RT), 30±2°C/65±5% RH and 40±2°C/75±5% RH in ICH certified stability chamber (Humidity Chamber, EIE Instruments Ltd., Ahmedabad, India) as per ICH guidelines (ICH Q1A(R2)). Nevirapine SEDDS (S1) equivalent to 50 mg was filled in a glass vial with a rubber closure and aluminium-crimped tops. Eight such glass vials were filled and stored at various aforementioned storage conditions up to 3 months. Samples were removed at 0, 1, 2 and 3 months of interval and checked for nevirapine content (by HPLC), droplet size, polydispersity index and zeta potential after diluting formulation to 100 times with three different media viz. Distilled water (DW), 0.1N HCl and phosphate buffer saline pH 6.8 (PBS) maintained at 37°C.

2.3. Results and discussion

2.3.1. Screening of oils and surfactants

Acceptable SEDDS formulation should be simple, safe, compatible and possess efficient droplet size after forming a microemulsion (Constantinides, 1995; Klous et al., 2004; Kommura et al., 2001; Subramanian et al., 2004). The appropriate vehicles should have good solubilizing capacity for the drug substance, which is essential for composing a SEDDS. The solubility of nevirapine in various vehicles is shown in Table 6. The components and their concentration ranges can be obtained by the construction of a pseudo-ternary phase diagram with constant drug level fixed at 4.5% (w/w). The drug loading capability is the main factor when screening the oil phase.

Nevirapine has the highest solubility in Caprylic acid as shown in Table 2.1 and was, therefore selected as an oil phase in the present study. On the basis of the solubility profile Soluphor® P was considered as a surfactant and Transcutol P as a co-surfactant.
Table 2.1: Solubility of nevirapine in various vehicles at 30°C (n=3)

<table>
<thead>
<tr>
<th>Vehicles</th>
<th>Solubility (mg/mL ± SD)</th>
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<tbody>
<tr>
<td>Oils</td>
<td></td>
</tr>
<tr>
<td>Oleic acid</td>
<td>12.22 ± 2.98</td>
</tr>
<tr>
<td>Caprylic acid</td>
<td>20.88 ± 2.84</td>
</tr>
<tr>
<td>Capryol 90</td>
<td>3.337 ± 1.09</td>
</tr>
<tr>
<td>Capmul MCM</td>
<td>6.095 ± 1.98</td>
</tr>
<tr>
<td>Surfactants</td>
<td></td>
</tr>
<tr>
<td>Cremophor EL</td>
<td>1.077 ± 0.47</td>
</tr>
<tr>
<td>Tween 20</td>
<td>2.76 ± 0.86</td>
</tr>
<tr>
<td>Tween 80</td>
<td>2.76 ± 0.86</td>
</tr>
<tr>
<td>Transcutol P</td>
<td>5.086 ± 2.04</td>
</tr>
<tr>
<td>Soluphor P</td>
<td>22.178 ± 3.46</td>
</tr>
</tbody>
</table>

2.3.2. Pseudo ternary phase diagrams

Phase diagrams of the systems containing Caprylic acid as an oil phase, Soluphor® P as a surfactant and Transcutol P as a co-surfactant were constructed at the surfactant/co-surfactant ratio of 1:4, 1:3, 1:2, 1:1 and 4:1 (w/w) to determine the existence of microemulsion region as shown in Figure 2.1. The phase study revealed that the obtained microemulsion regions at surfactant/co-surfactant ratios of 1:4 and 1:2 (Figure 2.1(a) and (c)) were similar with maximum of 4.65% oil incorporation. At surfactant/co-surfactant ratios of 1:3 and 1:1, about 4.88% of oil can be incorporated and gave the same microemulsion regions (Figure 2.1(b) and (d)). The ratio 4:1 of S/CoS showed maximum drug solubilisation capacity with 6% of oil incorporation (Figure 2.1(e)). It was observed that there was an improvement in the solubilisation of nevirapine with an increase in the proportion of the Soluphor® P. Based on our results, SEDDS formulation comprised of 8.56% oil Caprylic acid, 74.50% of Soluphor® P as surfactant and 16.93% of Transcutol P as co-surfactant. This selected composition of oil and surfactants should readily form microemulsion in the body on dilution with physiological fluids at 37°C.
Figure 2.1: Pseudo-ternary phase diagrams of the formulations composed of oil, surfactants and co-surfactant dispersed with distilled water at 37°C. Surfactants = Soluphor® P; Co-surfactant = Transcutol P. Surfactant to co-surfactant ratios (S/CoS) were as follows: (a) 1:4, (b) 1:3, (c) 1:2, (d) 1:1, (e) 4:1. The shaded area represents microemulsion region.

2.3.3. Characterization of self emulsifying drug delivery system

2.3.3.1. Drug content

Assay of prepared nevirapine SEDDS was carried out by UV-visible spectrophotometer. A linear calibration curve was obtained at 313 nm in the range of (8-30 µg/mL) with a correlation coefficient ($r^2$) of 0.999. Assay was found to be in the range of 100.9-105.1% with a standard deviation of ± 2.124 %.

2.3.3.2. Spectroscopic characterization of optical clarity

SEDDS was diluted with water to confirm the formation of microemulsion with the external phase of the system without phase separation. In order to assess the optical clarity quantitatively; UV-visible spectrophotometer was used to measure the transmitted light at 650 nm wavelength transmitted by the solution. Higher transmittance should be obtained with optically clear solutions, since cloudier solutions will scatter more of the incident radiation, resulting in lower transmittance. Aqueous dispersions with small absorbance are optically clear and oil droplets are thought to be in a state of finer dispersion. A clear o/w micro-emulsion was formed in both the dilution media. On 100 fold dilution percent
transmittance of the studied aqueous dispersion of nevirapine SEDDS was found to be 99.187 with distilled water and 98.158 with 0.1N HCl.

2.3.3.3. Morphological characterization

The TEM picture is shown in Figure 2.2. The microemulsion droplets were observed to be spherical and homogenous with large population of the smaller droplet in the size range of less than 200 nm. The droplet size of formed emulsion determined by TEM is in correlation with that of measured by photon correlation spectroscopy (PCS).

![TEM photo of NVP SEDDS S1 after dilution (x 43,000) (Scale: 500 nm)](image)

**Figure 2.2:** TEM photo of NVP SEDDS S1 after dilution (x 43,000) (Scale: 500 nm)

2.3.3.4. Determination of droplet size and zeta potential

Droplet size distribution following self-emulsification is a critical factor to evaluate a self-emulsifying system. The smaller the droplet size, the larger the interfacial surface area will be provided for drug absorption (Gershanik and Benita, 2000; Kang et al., 2004; Pouton, 2000). In our study, we investigated the effect of distilled water, 0.1N HCl and phosphate buffer saline pH 6.8 (PBS) as three different dilution media on droplet size. 0.1N HCl can be considered as a Simulated Gastric Fluid without pepsin, prepared according to USP. Pouton (1997) has proposed methods for the assessment of efficiency of self-emulsification using acidic medium (0.1N HCl) and water to simulate the range of conditions encountered in the GI tract. The average droplet size of microemulsion dispersed from the nevirapine SEDDS after 100 times dilution was found to be 180.2 nm in distilled water, 224.9 nm in 0.1N HCl and 1274 nm in phosphate buffer saline pH 6.8 (Figure 2.3 (a), (b) and (c)). The polydispersity of microemulsion dispersed from the nevirapine SEDDS was found to be 0.326, 0.456 and 1.0 in distilled water, 0.1N HCl and phosphate buffer saline pH 6.8, respectively. Zeta potential of the dispersed nevirapine
SEDDS in distilled water, 0.1N HCl and phosphate buffer saline pH 6.8 were -12.0 mV, 2.24 mV and -12.9 mV, respectively.

**Figure 2.3(a):** Droplet size distribution of nevirapine SEDDS S1 after dilution in distilled water

**Figure 2.3(b):** Droplet size distribution nevirapine SEDDS S1 after dilution in 0.1N HCl
2.3.3.5. In vitro diffusion study

*In vitro* diffusion study was performed to compare the drug release from the developed nevirapine SEDDS and conventional marketed suspension. Nearly $41.45 \pm 2.03\%$ of drug was released from nevirapine SEDDS within 1 hour compared to the marketed suspension which released $21.04 \pm 1.81\%$ of the drug. At the end of five hour period, almost all the drug ($98.18 \pm 2.81\%$) diffused from the SEDDS formulation compared to $65.13 \pm 2.98\%$ drug released from the conventional marketed suspension (Figure 2.4). Thus, the drug release from the nevirapine SEDDS was found to be significantly higher ($P < 0.05$) as compared to that of the marketed suspension. It could be suggested that the SEDDS formulation resulted in 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 nevirapine from the SEDDS formulation could lead to higher absorption and higher oral bioavailability.
Chapter 2  Nevirapine

Figure 2.4: *In vitro* diffusion profile of nevirapine SEDDS S1 and marketed suspension in 0.1N HCl ($n=6$)

2.3.3.6. *Ex-vivo* stomach permeability study

The profile of drug permeation across stomach is shown in Figure 2.5. At the end of 3 hours, 61.528% of drug was permeated across the stomach from developed nevirapine SEDDS compared to 49.066% from marketed suspension. The total percentage permeation from rat stomach was significantly higher ($P < 0.05$) for the nevirapine SEDDS than for the marketed suspension.

Figure 2.5: *Ex vivo* stomach permeability of nevirapine SEDDS S1 and marketed suspension in 100 mM HCl ($n=3$)
2.3.3.7. *Ex-vivo* intestinal permeability study

To understand the characteristics of drug permeation, *ex vivo* intestinal tissue permeation study was carried out across the small intestine of male Sprague-Dawley rats (Chen et al., 2008; Franceschinis et al., 2005; Smith, 1996; Yao et al., 2008). The amount of nevirapine permeated across rat intestinal tissue was determined by UV Spectrophotometer. A linear calibration curve was obtained at 283 nm in the range of (4-40 µg/mL) with a correlation coefficient ($r^2$) of 0.999. The profile of drug permeation is shown in Figure 2.6. In the first half hour, the permeation of drug through intestinal membrane from the nevirapine SEDDS and marketed suspension was 29.55 ± 5.16% and 27.22 ± 6.32%, respectively. More than 50% of the drug permeated across the intestine tissue within 2 hours from nevirapine SEDDS. On the other hand it took 3 hours for the marketed suspension. After 3 hours of permeation, more than 69% of the drug was accumulated from the SEDDS, as compared to 57% from the marketed suspension. The total percentage permeation from rat intestine was significantly higher ($P < 0.05$) for the nevirapine SEDDS than for the marketed suspension.

![Ex vivo intestinal permeability study of Nevirapine SEDDS and Marketed Suspension](image)

**Figure 2.6**: *Ex vivo* intestinal permeation profile of nevirapine SEDDS S1 and marketed suspension in pH 6.8 PBS ($n=3$)

The main mechanism reported to improve the oral absorption of lipophilic drugs when incorporated into SEDDS include, increasing membrane fluidity, opening of tight cellular junctions, inhibiting P-gp and/or CYP450 by surfactants and stimulating lipoprotein/chylomicron production by lipid (Humberstone and Charman, 1997; Wu et al.,...
The better permeation observed with the developed nevirapine SEDDS formulation might be due to the higher surfactant content that could have made the intestinal wall more permeable by partial disruption of membrane. Oral absorption of the SEDDS and micelles showed that the surfactant-induced membrane fluidity might play an important role in the permeability change and the increase of drug absorption in the gastrointestinal tract (Kommura et al., 2001; Sha et al., 2005).

### 2.3.4. HPLC method development and validation

The method was found to be specific, as IS and NVP were well resolved and no interfering peaks from endogenous components of normal plasma were observed (Figure 2.7(a) and 2.7(b)). The method was found to be linear over the range 25-5000 ng/mL (Figure 2.8). The calibration ranges were selected based on the expected concentration in bioavailability study samples. The least square linear regression analysis with weighing factor 1/x of the peak areas ratios of nevirapine and IS (y) versus concentration of nevirapine in plasma (x), obtained by assaying plasma sample spiked with nevirapine with correlation coefficient values above 0.99 is shown in Table 2.2.

![Figure 2.7(a): A representative HPLC chromatogram obtained for blank plasma used for preparing standards and quality control samples of nevirapine](image-url)
Figure 2.7(b): A representative chromatogram of plasma spiked with standard nevirapine and Carbamazepine (IS) for standards and quality control samples of nevirapine.

Figure 2.8: Calibration curve of nevirapine in rat plasma.
Table 2.2: Linear regression analysis of calibration curve for nevirapine spiked in plasma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slope</th>
<th>Intercept</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>0.0009</td>
<td>0.0056</td>
<td>0.99953</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.0009</td>
<td>0.0070</td>
<td>0.99944</td>
</tr>
<tr>
<td>Day 3</td>
<td>0.0009</td>
<td>0.0054</td>
<td>0.99987</td>
</tr>
</tbody>
</table>

The LLOQ was 25 ng/mL. Sensitivity of the method is the lowest concentration that can be measured with an acceptable limit of accuracy and precision. The accuracy and precision at LLOQ was determined by analyzing at least 5 replicates of the sample at the LLOQ concentration on at least one of the validation days. These samples were independent of those used for construction of the calibration curve. All the results of sensitivity samples were also used to calculate accuracy and precision. The intra-day accuracy at LLOQ (25 ng/mL) was found to be 100.54 % with precision of 2.90 % (%RSD) (Table 2.3). The values are within the limits of specified in the guidelines of bioanalytical method validation (ICH Q2(B)). Accuracy should be ± 20% and the RSD should be less than 20% at LLOQ.

Table 2.3. Intra-day accuracy and precision (%RSD) of 5 determinations for estimation of nevirapine in rat plasma on the same day.

<table>
<thead>
<tr>
<th>Nevirapine concentration in ng/mL</th>
<th>Mean</th>
<th>LLOQ</th>
<th>LQC</th>
<th>MQC</th>
<th>HQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td>25.135</td>
<td>70.938</td>
<td>502.044</td>
<td>3637.67</td>
</tr>
<tr>
<td>SD</td>
<td>0.73</td>
<td>3.63</td>
<td>3.34</td>
<td>85.21</td>
<td></td>
</tr>
<tr>
<td>%RSD a</td>
<td>2.90</td>
<td>5.12</td>
<td>0.66</td>
<td>2.34</td>
<td></td>
</tr>
<tr>
<td>Accuracy b</td>
<td>100.54</td>
<td>94.58</td>
<td>100.40</td>
<td>103.93</td>
<td></td>
</tr>
</tbody>
</table>

\[ ^a \text{%RSD} = \frac{SD}{Mean} \times 100 \]

\[ ^b \text{Accuracy} = \frac{\text{Concentration found}}{\text{actual concentration}} \times 100 \]

Accuracy and precision of the analytical method was determined for both intra- and inter-runs. They were determined by analyzing quality control (QC) samples at a minimum of 3 concentrations (low, mid and high), representing the entire range of the calibration curve (25-5000 ng/mL). As the concentration of low QC (LOQ) should be near the lower limit of quantification and should not be more than 3 times the LLOQ concentration and hence 75 ng/mL was selected as LQC. The mid QC concentration should be somewhere in the middle of the calibration range and hence 500 ng/mL was selected as MQC. The high QC concentration should be near the upper end of the calibration curve (within the upper quartile of the calibration range). HQC selected was 3500 ng/mL. At least five replicates
at each concentration were analyzed. The results of the intra-day accuracy and precision are shown in Table 2.3 and inter day accuracy and precision data is shown in Table 2.4.

**Table 2.4.** Inter-day accuracy and precision (%RSD) of 3 determinations for estimation of nevirapine in rat plasma on the same day.

<table>
<thead>
<tr>
<th>Nevirapine concentration in ng/mL</th>
<th>LLOQ</th>
<th>LQC</th>
<th>MQC</th>
<th>HQC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>25.61</td>
<td>72.56</td>
<td>501.24</td>
<td>3677.83</td>
</tr>
<tr>
<td>Day 2</td>
<td>26.76</td>
<td>75.29</td>
<td>508.75</td>
<td>3579.86</td>
</tr>
<tr>
<td>Day 3</td>
<td>25.48</td>
<td>74.74</td>
<td>506.4</td>
<td>3510.14</td>
</tr>
<tr>
<td>Mean</td>
<td>25.95</td>
<td>74.20</td>
<td>505.46</td>
<td>3589.28</td>
</tr>
<tr>
<td>SD</td>
<td>0.70</td>
<td>1.44</td>
<td>3.83</td>
<td>84.24</td>
</tr>
<tr>
<td>%RSD a</td>
<td>2.71</td>
<td>1.94</td>
<td>0.75</td>
<td>2.34</td>
</tr>
<tr>
<td>Accuracy b</td>
<td>103.8</td>
<td>98.93</td>
<td>101.09</td>
<td>102.55</td>
</tr>
</tbody>
</table>

*a. %RSD = SD/Mean * 100

*b. Accuracy = Concentration found/actual concentration * 100

The method precision (%RSD) at LLOQ were 4.13 % (intraday) and 2.86 % (interday) and accuracy of 101.9 % and 103.62 % for nevirapine, intra- and inter-day respectively (Table 2.3 and 2.4). As per the bioanalytical method validation guidelines (ICH Q2 (B)), the mean of the observed concentration should be within ± 15% of the nominal at all concentrations of the QC samples. Coefficient of variation (indication of precision) around the mean observed concentration should not exceed 15% at all concentration, and the CV should be less than 15%, at all concentrations. If the QC concentration is at the lower limit of quantification, the RE% at the CV can be up to 20%.

Stability of the nevirapine in plasma samples was observed for three freeze-thaw cycles is reported in Table 2.5.

**Table 2.5.** Stability of nevirapine in rat plasma

<table>
<thead>
<tr>
<th>Sample concentration (ng/mL)</th>
<th>Stability sample concentration d (ng/mL)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bench top a</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQC (75)</td>
<td>74.03 ± 1.25</td>
<td>98.72</td>
</tr>
<tr>
<td>HQC (3500)</td>
<td>3655 ± 48.92</td>
<td>104.4</td>
</tr>
<tr>
<td><strong>Freeze-thaw b</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQC (75)</td>
<td>73.67 ± 0.59</td>
<td>98.22</td>
</tr>
<tr>
<td>HQC (3500)</td>
<td>3704 ± 28.46</td>
<td>105.83</td>
</tr>
<tr>
<td><strong>Post preparative c</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQC (75)</td>
<td>74.39 ± 0.96</td>
<td>99.19</td>
</tr>
<tr>
<td>HQC (3500)</td>
<td>3805 ± 77.8</td>
<td>108.73</td>
</tr>
</tbody>
</table>

*a. After 6 h at room temperature

*b. After 3 freeze-thaw cycles

*c. After 24 h at 25°C in Autosampler

*d. Mean ± SD (n=3)

Reconstituted organic extracts were stable for 24 h in the autosampler at 25 ° C as indicated by post preparative stability in Table 2.5. The spiked plasma samples as well as
the nevirapine stock solutions were stable for a minimum of 6 h at room temp (Table 2.5). The dried extracts of samples showed stability when left overnight at -80°C.

The validation results of bioanalytical method for estimation of nevirapine in rat plasma indicate that the method is simple, specific, precise and accurate and suitable for estimation of NVP from plasma samples of bioavailability studies in rats.

2.3.5. Bioavailability study

Typical chromatograms obtained showing peaks for NVP at 1 h after administration of marketed suspension and SEDDS are shown in Figure 2.9(a) and 2.9(b), respectively. The mean pharmacokinetic parameters of SEDDS and marketed conventional suspensions were compared in rats.

![Figure 2.9(a): Typical chromatogram obtained while analyzing plasma for NVP level after administration of the marketed suspension at 1 h.](image-url)
Figure 2.9(b): Typical chromatogram obtained while analyzing plasma for NVP level after administration of the SEDDS S1 at 1 h.

Figure 2.10 shows the plasma profiles of nevirapine after oral administration of the two preparations. The pharmacokinetic parameters are given in Table 2.6. The $C_{\text{max}}$, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow \infty}$ of the SEDDS were significantly higher ($P < 0.05$) than those of the suspension. The relative bioavailability of nevirapine SEDDS was approximately 2.69-fold when compared with the marketed suspension. Therefore, SEDDS might be a promising approach for the oral delivery of nevirapine.

Figure 2.10: Plasma concentration profile of nevirapine after oral administration of SEDDS and marketed suspension in rats ($n=4$ and 20 mg/kg)
Table 2.6: Relative bioavailability and pharmacokinetic parameters of nevirapine after oral administration of nevirapine SEDDS and marketed suspension to the rats (n=4)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Marketed Suspension</th>
<th>SEDDS (S1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{0\rightarrow t}$ (ng.h/mL)</td>
<td>7082.736 ± 1941.31</td>
<td>18996.62 ± 5553.135</td>
</tr>
<tr>
<td>$AUC_{0\rightarrow \infty}$ (ng.h/mL)</td>
<td>7422.146 ± 2100.749</td>
<td>20020.64 ± 4996.968</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>1509.99 ± 517.602</td>
<td>4077.491 ± 2157.834</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.0 ± 0.0</td>
<td>1.25 ± 0.5</td>
</tr>
<tr>
<td>Relative bioavailability (%)</td>
<td>---</td>
<td>269.74</td>
</tr>
</tbody>
</table>

Soluphor $^\text{®}$ P has been much explored as permeation enhancer for topical and parenteral application. In recent years, Soluphor $^\text{®}$ P has been tried by many researcher groups for solubility and permeability improvement of poorly-soluble drugs for oral human use. Shende et al. (2007) were successful to solubilise Lamotrigene, an anti-epileptic drug, by virtue of microemulsion for nasal delivery using Soluphor $^\text{®}$ P as a co-surfactant. Dharap et al (2005) tried several solvents and solubilizers to solubilise Ibuprofen and Nifedipine and reported that Soluphor $^\text{®}$ P and Solutol $^\text{®}$ HS15 (Polyoxyl 15 hydroxystearate) improved solubility and were compatible for use in hard gelatine capsules. Ruchatz and Kotler, (1998) studied the properties of 2-pyrrolidone (Soluphor $^\text{®}$ P) as a co-solvent for the dissolution enhancement of water insoluble drugs like Clotrimazole, 17-$\beta$-estradiole, Sulfathiazole, Riboflavin, PHB-ester, Acetaminophen in comparison to other commonly used solvents. It was found that the efficacy of Soluphor $^\text{®}$ P was considerably higher than other often used co-solvents. Our study too indicated that among the various surfactants and solubilizers, Soluphor $^\text{®}$ P has shown the maximum solubility of nevirapine.

The in vitro diffusion study across dialysis membrane and ex vivo intestinal permeation study across intestine of male wistar rat showed better results ($P < 0.05$) compared to marketed conventional suspension. The present investigation of in vivo absorption in animal model confirms the solubilising and permeation enhancing property of Soluphor $^\text{®}$ P.

2.3.6. Stability Studies

No change in the physical parameters such as homogeneity and clarity was observed during the stability studies. Droplet size, polydispersity index and zeta potential remained unchanged even after keeping the formulations at different environmental storage conditions for 3 months, indicating the physical stability of developed formulations (Table 2.7). No decline in the drug content was observed at the end of 3 months indicating that drug remained chemically stable in SEDDS.
Table 2.7: Stability studies of nevirapine SEDDS S1 at various storage conditions

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Parameters</th>
<th>Medium 1</th>
<th>25 ± 2°C (RT)</th>
<th></th>
<th>30 ± 2°C/65 ± 5% RH</th>
<th></th>
<th>40 ± 2°C/75 ± 5% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Droplet Size D (nm)</td>
<td>DW</td>
<td>185.7</td>
<td>189.3</td>
<td>193.31</td>
<td>180.35</td>
<td>186.5</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>227.67</td>
<td>200.01</td>
<td>234.47</td>
<td>239.17</td>
<td>225.2</td>
<td>204.95</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>1294</td>
<td>1244</td>
<td>1298</td>
<td>1307</td>
<td>1296</td>
<td>1253</td>
</tr>
<tr>
<td></td>
<td>Polydispersity index (PdI)</td>
<td>DW</td>
<td>0.348</td>
<td>0.336</td>
<td>0.325</td>
<td>0.388</td>
<td>0.321</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>0.448</td>
<td>0.484</td>
<td>0.438</td>
<td>0.448</td>
<td>0.495</td>
<td>0.485</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>2.64</td>
<td>2.25</td>
<td>3.62</td>
<td>3.21</td>
<td>2.22</td>
<td>2.74</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>-13.02</td>
<td>-14.02</td>
<td>-14.02</td>
<td>-14.02</td>
<td>-14.02</td>
<td>-14.02</td>
</tr>
<tr>
<td></td>
<td>Drug Content (%)</td>
<td>102.59</td>
<td>100.48</td>
<td>102.2</td>
<td>100.9</td>
<td>102.54</td>
<td>99.45</td>
</tr>
</tbody>
</table>

D (nm) = diameter in nanometer; DW = distilled water; PBS = phosphate buffer saline pH 6.8; RT = room temperature; M = month; RH = relative humidity

2.4. Conclusion

A SEDDS containing poorly water-soluble drug, nevirapine, was formulated for oral administration. The components and their ratio ranges for the formulation of SEDDS were obtained by solubility study, pseudo-ternary phase diagram construction, and droplet size analysis. The optimum formulation of the SEDDS consisted of 8.56% of Caprylic acid as oil, 74.50% of Soluphor® P as surfactant and 16.93% of Transcutol P as co-surfactant, which had sufficient drug loading, rapid self-emulsification in aqueous media, and forming droplet size in the range of microemulsion. Our study indicated that the developed nevirapine SEDDS formulation showed greater diffusion and stomach and intestinal permeability than the marketed suspension. The pharmacokinetic data indicates that the nevirapine SEDDS have better in vivo absorption compared to marketed suspension. The higher bioavailability might be due to the enhanced solubility of nevirapine and the composition of the delivery system. The investigation also supports the wide application of Soluphor® P including dermal and parenteral in pharmaceuticals for poorly water-soluble drugs. In conclusion, the data obtained in the present study highlight the importance of medium chain triglycerides and solubilizer in the enhancement of lipophilic drug by self-emulsifying drug delivery system. Despite the fact that MCT are absorbed primarily by portal route and nevirapine being extensively metabolised, our results suggest that the combined effect of rapid hydrolysis and efficient absorption of caprylic acid and permeation enhancing property of Soluphor® P may be responsible for improved bioavailability. It seems that oil component in the formulation may be sufficient enough to trigger endogenous release of triglycerides and re-synthesis of medium chain triglycerides.
to triacylglycerol for formation of chylomicrons and subsequently intestinal lymphatic absorption (Caliph et al., 2000; Shiau et al., 1985). However, a further investigation is needed for these observations. The results of the study suggest that both lymphatic transport and absorption via portal blood of nevirapine can be enhanced after co-administration of structured triglyceride vehicle comprising both medium- and long-chain fatty acids. The structured lipid may also provide additional pharmaceutical benefits in the solubility of lipophilic drugs such as nevirapine. As medium chain fatty acids are absorbed into intestinal cells without micellar solubilisation in intestinal lumen, the amount of surfactant/solubilizer may be reduced and will be more compliant for HIV/AIDS patients with debilitated gastrointestinal tract. More work needs to be done in this area to assess the potential of medium chain triglycerides in development of lipid-based antiretroviral therapy for AIDS patients.
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Chapter 2

Nevirapine


