Chapter 3
Formulation and evaluation of self-emulsifying formulations of nevirapine using pharmaceutically accepted lipid-based excipients
Page no. 89 to 115
Chapter 3

Formulation and evaluation of self-emulsifying formulations of nevirapine using pharmaceutically accepted lipid-based excipients

Abstract

The objective of this study was to develop self-emulsifying drug delivery system (SEDDS) to improve solubility and enhance the oral absorption of the poorly water-soluble drug, nevirapine. This lipid-based formulation may help to target the drug to lymphoid organs where HIV-1 virus resides mainly. The influence of the oil, surfactant and co-surfactant types on the drug solubility and their ratios on forming efficient and stable SEDDS were investigated in detail. Two SEDDS (F1 and F2) were prepared and characterized by morphological observation, droplet size and zeta potential determination, cloud point measurement and in vitro diffusion study. The influence of droplet size on the absorption from formulations with varying concentration of oil and surfactant was also evaluated from two self-emulsifying formulations. Oral bioavailability of nevirapine SEDDS was checked by using rat model. Results of diffusion rate and oral bioavailability of nevirapine SEDDS were compared with marketed suspension. Diffusion of nevirapine F2 showed maximum drug release when compared to F1 and marketed suspension. The absorption of nevirapine from F1 and F2 showed 1.92- and 1.98-fold increase ($P < 0.05$) in relative bioavailability, respectively compared with that of the suspension. There was no statistical significant difference ($P < 0.05$) between F1 and F2 in their $AUC$ and $C_{\text{max}}$. This indicated that there was apparent poor correlation between the droplet size and in vivo absorption. However, nevirapine in SEDDS showed higher ex vivo stomach and intestinal permeability and in vivo absorption than the marketed suspension, suggesting that the SEDDS may be a useful delivery system for targeting nevirapine to lymphoid organs.
3.1. Introduction

Nevirapine is a poorly water-soluble drug and is currently available as a tablet and in pediatric oral suspensions. In this study we demonstrate the development of self-emulsifying drug delivery system employing widely used and accepted lipid-based excipients. The present investigation also describes the detailed procedures for characterisation of SEDDS with help of reported techniques.

A self-emulsifying formulation has an ability to form fine colloidal droplets with very high surface area. In many cases, this accelerates the digestion of the lipid formulation, improves absorption, and reduces food effect and inter-subject variability (Constantinides, 1995; Humberstone and Charman, 1997; Pouton, 1997, 2000).

The self-emulsification process was shown to be dependent on the lipid/surfactant pair, the surfactant concentration, the ratio between lipid and surfactant (Pouton, 1985; Wakerly et al., 1987). However, only specific combinations can lead to an efficient self-emulsifying system (Charman et al., 1992; Shah et al., 1994). It is advantageous to use medium chain triglycerides (MCT) SEDDS due to their higher fluidity, better solubility properties and self-emulsification ability compared with long chain triglycerides (LCT) (Charman et al., 1992; Shah et al., 1994), as well as a better chemical stability of drug substance in MCT due to the purity of the lipid and the lack of double bonds, that can catalyse oxidation. The two lipids are differently transported in the body: MCT is directly transported by the portal blood to the systemic circulation (Porter and Charman, 1997), whereas LCT is transported in the intestinal lymphatics. However, some of the medium-chain saturated fatty acids are resynthesized or re-esterified to triacylglycerols or neutral lipids, packaged within apoprotein surface layers and transported via the lymphatic system (Carvajal et al., 2000; Green and Glickman, 1981; Holm et al., 2002; Ikeda et al., 1991; Mu and Hoy, 2001).

Recently, Sun et al (2011) have reported that, the lymphatic transport of sirolimus from SMEDDS formulation containing $\geq$ 25% MCT was a major contributor to its oral bioavailability.

HIV/AIDS patients often have severe fat malabsorption and thus their quality of life is greatly diminished. If they could ingest a diet containing a readily absorbable fat source, such as MCTs, their steatorrhea and stool nitrogen excretion could be diminished and their nutritional status may be improved. (Craig et al., 1997; Wanke et al., 1996). Craig et al (1997) have demonstrated that the MCT-containing formula to be better tolerated by AIDS
patients, with fat malabsorption, compared to control liquid formulas (relative to solid food diet) and suggested as a source of dietary fat for such patients.

The objectives of the present study were: (1) To develop and optimize the self emulsifying formulation of nevirapine; (2) To assess its properties in vitro; (3) To investigate the influence of droplet size of self emulsifying formulation on drug absorption; and (4) To assess its oral bioavailability compared with a conventional dosage form using animal model.

The aim of the present study was to evaluate the potential of self emulsifying drug delivery system to improve the absorption of dissolution-rate limited bioavailability of nevirapine, using traditional and pharmaceutically accepted lipid-based excipients. The effect of surfactant:oil ratios on droplet size and in vivo absorption was also studied in rat.

3.2. Materials and methods

3.2.1. 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) was obtained from BASF Corp. (Mumbai, India). Diethylene glycol monoethyl ether (Transcutol P®), PEG-8 glycol caprylate (Labrasol®), Propylene glycol monocaprylate (Capryol 90®) were provided by Gattefosse, France and Glycerol Monocapryloocaprate (Capmul MCM®) was provided by Abitec Corp., Janesville WI. PEG-400, Tween 20, Tween 80, Propylene glycol 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.

3.2.2. Solubility studies

The solubility of nevirapine in various oils, surfactants and co-surfactants was determined. 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 nstruments Ltd., Ahmedabad, India). Mixtures were equilibrated at 30°C for 48 h in a water bath and then centrifuged in a laboratory centrifuge (Biofuge, Thermo Electron Corp. USA) 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).

3.2.3. Pseudoternary Phase Diagrams

Pseudoternary phase diagrams of oil, surfactant/ co-surfactant (S/CoS), and water were developed using the water titration method (Djordjevic et al., 2004). The mixtures of oil and S/CoS at certain weight ratios (9:1 to 1:9 w/w) were diluted with water in a drop wise manner. For each phase diagram at a specific ratio of S/CoS (i.e., 2:1, 1:1 and 1:2 w/w), a transparent and homogenous mixture of oil and S/CoS was formed by vortexing for 5 minutes. Then each mixture was titrated with water and visually observed for phase clarity and flowability. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occurred was derived from the weight measurements. These values were then used to determine the boundaries of the microemulsion domain corresponding to the chosen value of oils, as well as the S/CoS mixing ratio. Phase diagrams were then constructed using Tri plot v1.4 software (David Graham and Nicholas Midgley, Loughborough, Leicestershire, UK). Following studies were carried out by constructing a ternary phase diagram: (1) the influence of the ratio of surfactant to co-surfactant on the formation of microemulsion. (2) the effect of more than one surfactant in different ratios with Transcutol P as co-surfactant. (3) the influence of surfactants on microemulsion formation with Transcutol P as co-surfactant

3.2.4. Preparation of self-emulsifying systems

The formulations consisted of nevirapine: Caprylic acid: (Labrasol:Tween 80, 1:3):Transcutol P (2.5:19.5:62.4:15.6 and 2.5:9.75:75.22:12.54) (% w/w). The formulations were prepared by dissolving the formulation amount of nevirapine in the mixture of Transcutol P and Caprylic acid at 50°C in an isothermal water bath (Equitron, Chennai, India). Labrasol and Tween 80 were then added. This mixture was mixed by vortexing until a transparent preparation was obtained.

3.2.5. Characterization of Self emulsifying formulations

3.2.5.1. Drug content

SEDDS formulation equivalent to 25 mg of nevirapine was taken and diluted in methanol. Volume was made up to 25 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.

3.2.5.2. 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 43,000×. 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.

3.2.5.3. Determination of droplet size and zeta (ζ) potential

Droplet size and the zeta potential of the formed emulsion were determined by photon correlation spectroscopy 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. All the measurements were performed at room temperature (25°C) in triplicate.

3.2.5.4. 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; 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 clip (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 nevirapine present in the dialyzing medium at corresponding time by UV-visible spectrophotometer (Shimadzu UV-2450, Japan) at 313 nm.
3.2.5.5. *Ex vivo* stomach permeability

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 formulations, 0.2 mL (equivalent to 5 mg of drug) were 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. 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.

3.2.5.6. *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, 1.04 g
(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.

3.2.6. HPLC analysis

See Chapter 2 Sec. 2.2.6. HPLC method development and validation.

3.2.7. 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. Three 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 (F1 and F2) and marketed suspension (Nevimune®)) 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 & Barnes, 1964). The animals were fasted overnight prior to the experiment but had free access to water. The developed SEDDS and marketed suspension (Nevimune®) were
administered by oral snode in an equivalent dose of 20 mg/kg of nevirapine. 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, 1, 2, 4, 8, 12, 16 and 24 hours after oral administration. The blood samples were collected into a heparinized microcentrifuge tubes (13 x 75mm, 2mL, Accuvac, BD, NJ), subjected to centrifugation on a laboratory centrifuge and supernatant plasma was collected and kept at –20°C (Thermo Scientific, USA) until analysis.

3.2.7.1. 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 a clean tube and dried under nitrogen stream at ambient temperature. The residue was reconstituted with a 100 μL aliquot of mobile phase, consisted of 10 mM Sodium phosphate pH 5.0 and ACN (70:30 v/v) 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$_{0\rightarrow t}$) from zero to 24 hour was estimated by the linear trapezoidal method (Han and Lee, 1999; Lee and Ku, 1999). The relative bioavailability ($F$) of SMEDDS to the suspensions was calculated using the following equation:

$$F = \frac{(AUC_{\text{test}})}{(AUC_{\text{reference}})} \times 100\%$$

3.2.8. 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.
3.2.9. Stability studies

Chemical and physical stability of nevirapine SEDDS (F1 and F2) were assessed under various storage conditions namely room temperature (RT), 30±2°C/65±5% RH and 40±2°C/75±5% RH as per ICH guidelines (ICH Q1A(R2)) in ICH certified stability chambers (Humidity Chamber, EIE Instruments Ltd., Ahmedabad, India).

Nevirapine SEDDS equivalent to 25 mg was filled in a glass vial with a rubber closure and aluminium-crimped tops. Eight such glass vials of each F1 and F2 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.

3.3. Results and discussion

3.3.1. Screening of oils and surfactants

The results of solubility of nevirapine in few vehicles are shown in Table 3.1. Solubility studies were carried out to identify potential ingredients for the formation of microemulsion. Amongst the various oils that were screened, Caprylic acid exhibited highest solubilising potential for nevirapine and was selected as an oily phase for further studies (Table 3.1). Amongst various surfactants, Labrasol exhibited good solubilising potential for nevirapine. However, Labrasol has shown relatively poor microemulsifying properties as compared to other hydrophilic surfactants. Preliminary studies indicated that a mixture of Labrasol and Tween 80 showed reasonable solubilising potential for nevirapine and also better emulsifying properties when evaluated by method reported by Date and Nagarsenkar (2007). The components and their concentration ranges can be obtained by construction of a pseudo-ternary phase diagram with constant drug level fixed at 2.5% (w/w). The drug loading capability is the main factor when screening the oil phase.

Labrasol has shown better solubility but poor microemulsion region compared to Tween 80, hence in early stage Labrasol alone was considered as a surfactant. As to the selection of co-surfactant, Transcutol P showed better solubility in the presence of Labrasol than PEG-400 and propylene glycol. Therefore it is reasonable to select Transcutol P as the co-surfactant.
Table 3.1: Solubility of nevirapine in various vehicles at 30°C (n=3)

<table>
<thead>
<tr>
<th>Vehicles</th>
<th>Solubility (mg/mL ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oils</strong></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><strong>Surfactants</strong></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>Labrasol</td>
<td>12.702 ± 2.09</td>
</tr>
<tr>
<td>PEG-400</td>
<td>8.68 ± 1.36</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>5.547 ± 2.04</td>
</tr>
</tbody>
</table>

Visual observation and percent transmittance data proved that Labrasol when used alone as a surfactant is unable to incorporate large amount of water compared to Tween 80. But the combination of these two surfactants showed enhanced water uptake capacity. Among the 1:1, 1:2 and 1:3 ratios of Labrasol: Tween 80; 1:3 showed better solubility and dilution potential. Hence the mixture of Labrasol and Tween 80 in the ratio of 1:3 was selected as a surfactant.

Phase diagrams of the systems containing Caprylic acid as an oil phase, mixture of Labrasol and Tween 80 as a surfactant and Transcutol P as a co-surfactant (CoS) were constructed at the surfactant (Labrasol: Tween 80; 1:3 (w/w))/co-surfactant ratio of 2:1, 1:1 and 1:2 (w/w) and shown in Figure 3.1. Maximum microemulsifying region was found to be at the surfactant/co-surfactant in the ratio of 1:2 followed by 2:1 having surfactant mixture ratio of 1:3 (Labrasol:Tween 80).
Figure 3.1: Pseudo-ternary phase diagrams of the formulations composed of oil, surfactants and co-surfactant dispersed with distilled water at 37 °C. Surfactants = Labrasol: Tween 80, 1:3; Co-surfactant = Transcutol P. Surfactant to co-surfactant ratios were as follows: (a) 2:1, (b) 1:1, (c) 1:2. The shadow area represents microemulsion region.

Usually Transcutol P is used as co-surfactant in less concentration compared to surfactants. Keeping this in mind, surfactant/co-surfactant in the ratios of 6:1, 4:1 and 2:1 with surfactant mixture 1:3 (Labrasol:Tween 80) were tried for formulation preparations in the ratios of oil:S\textsubscript{mix} of 1:4, 3:7 and 1:9 and were characterised for percent transmittance and globule size after 100 times dilution. The percent transmittance was in the range of 91.69-100.50 when Oil: S\textsubscript{mix} ratio was 1:9. The droplet size increases as the percent transmittance falls. The percent transmittance improved in the order of S/CoS ratio 6:1 > 4:1 > 2:1 > 1:2. There was decrease in the percent transmittance and increase in the average droplet size in the order of Oil: S\textsubscript{mix} ratio 1:9 < 1:4 < 3:7. The influence of different ratios of surfactant [Labrasol: Tween 80(1:3)] to co-surfactant (Transcutol P) on droplet size was also investigated.

Based on our results, two SEDDS formulations, F1 and F2 were established: a) F1 comprised of 20% of Caprylic acid as oil, 64% mixture of Labrasol and Tween 80 (1:3) as surfactant and 16% Transcutol P as co-surfactant. b) F2 comprised of 10% Caprylic acid as oil, 77.14% mixture of Labrasol and Tween 80 (1:3) as surfactant and 12.86% Transcutol P as co-surfactant.
3.3.2. SEDDS characterization

3.3.2.1. Morphological characterization

The nevirapine SEDDS turned into transparent emulsion when diluted with distilled water. The TEM pictures were shown in Figure 3.2(a) and Figure 3.2(b)). The formed emulsion droplets were observed to be spherical.

![TEM photo of nevirapine microemulsion (x 43,000) (Scale: 500 nm) TEM of (a) NVP F1 and (b) NVP F2](image)

**Figure 3.2:** TEM photo of nevirapine microemulsion (x 43,000) (Scale: 500 nm) TEM of (a) NVP F1 and (b) NVP F2

3.3.2.2. Determination of droplet size and $\zeta$-Potential

The average droplet size of microemulsion dispersed from the nevirapine SEDDS was within 260 nm and 100 nm when Oil:S$_{\text{mix}}$ ratio was 1:4 and 1:9, respectively and showed Gaussian distribution.

The effect of medium on droplet size was also investigated in our study. When the SEDDS dispersed in distilled water, 0.1N HCl and pH 6.8 phosphate buffer, the resulted droplet size was $210.8 \pm 0.56$ nm, $223.34 \pm 0.48$ nm and $163.1 \pm 0.66$ nm, respectively for Oil:S$_{\text{mix}}$ 1:4 and S/CoS 4:1 (F1) (Figure 3.3 (a), (b), (c)).
Figure 3.3 (a): Droplet size distribution of nevirapine SEDDS F1 after dilution in distilled water

Figure 3.3 (b): Droplet size distribution of nevirapine SEDDS F1 after dilution in 0.1N HCl
When Oil:S\text{mix} ratio was 1:9 and S/CoS 6:1 (F2), the resulted droplet size after dispersing in distilled water, 0.1N HCl and pH 6.8 phosphate buffer was 14.63 ± 0.39 nm, 14.25 ± 0.35 nm and 13.17 ± 0.39 nm, respectively (Figure 3.4 (a), (b), (c)).
Generally, an increase of electrostatic repulsive forces between microemulsion droplets prevents the coalescence of microemulsion droplets. On the contrary, a decrease of electrostatic repulsive forces will cause phase separation. Nevirapine SEDDS was diluted with distilled water, 0.1N HCl and pH 6.8 phosphate buffer saline, and the resulted zeta potential was $-5.2 \pm 0.35\text{mV}$, $2.74 \pm 0.17\text{mV}$, and $-2.73 \pm 0.18\text{mV}$, respectively for F1 and $-6.02 \pm 0.54\text{mV}$, $15.2 \pm 0.49\text{mV}$, $-4.27 \pm 0.21\text{mV}$, respectively for F2. It can be seen that
surface charge was positive for the droplets in 0.1N HCl, which indicated that these formulations would reach a positive zeta potential at physiological pH. Several studies have reported that the zeta potential played an important role in the interactions with mucus of the gastrointestinal tract (Gershanik et al., 2000; Wei et al., 2005). According to the reports, the positive charged droplets could have better interaction with the mucus of the gastrointestinal tract, since the intestinal cell interior carry negative charges with the presence of mucosal fluid.

3.3.2.3. In vitro diffusion study

In vitro diffusion data are shown in Figure 3.5. The rate of drug diffusion was in the order of F2 > marketed suspension > F1. The total percentage diffusion was higher for the F2 SEDDS than for the suspension dosage form. After 5 hours of diffusion, more than 70% of the drug was diffused from the self-emulsifying system, as compared with 64% diffused from the marketed suspension. The solubility of nevirapine increases as pH drops, therefore, the initial release profile of all the three formulations looks alike in 0.1N HCl.

![In vitro diffusion study of NVP SEDDS and marketed suspension](image)

**Figure 3.5:** In vitro diffusion profile of nevirapine SEDDS and marketed suspension in 0.1N HCl \((n=6)\)

3.3.2.4. Ex-vivo stomach and intestinal permeability study

The profile of drug permeation across stomach is shown in Figure 3.6. At the end of 3 hours, 49.86% and 56.42% of drug was permeated across the stomach from developed nevirapine SEDDS, F1 and F2 respectively, compared to 49.066 % from marketed suspension. The total percentage permeation from rat stomach was significantly higher \((P < 0.05)\) for the nevirapine SEDDS F2 than for the marketed suspension. The weakly basic
drug nevirapine has better solubility in acidic condition and this could be the reason for similar stomach permeation profile. On the other hand, SEDDS F1 is composed of about 20% of oil and it needs some time to breakdown the oil globules before releasing the drug. SEDDS F2 which contains 10% oil, has higher proportion of surfactant compared to F1 and may be responsible for better release of nevirapine in an acidic medium.

**Figure 3.6:** *Ex vivo* stomach permeability of nevirapine SEDDS and marketed suspension in 100 mM HCl (*n*=3)

The profile of intestinal drug permeation is shown in Figure 3.7. At the end of 5 hours, the percentage of nevirapine permeated across rat intestinal tissue was 62.88 ± 1.43, 71.03 ± 1.08 and 57.00 ± 6.77 for F1, F2 and markets suspension, respectively. More amount of oil needs to be broken down to release the drug from the oil globule as seen in F1. The initial similar permeation profile of marketed suspension to that of developed SEDDS may be due to dissolved and smaller drug particles of suspension, and also the availability of the fresh medium. The drug permeation (both Stomach and intestinal permeability) from the nevirapine SEDDS formulation F2 was found to be significantly higher (*P* < 0.05) as compared to that of the marketed suspension and F1. But there was no significant difference in the permeation of nevirapine SEDDS formulation F1 and marketed suspension at *P* < 0.05.
Figure 3.7: *Ex vivo* intestinal permeability of nevirapine SEDDS and marketed suspension in pH 6.8 PBS (n=3)

3.3.3. HPLC analysis

As discussed in Chapter 2 Sec. 2.3.4. HPLC method development and validation

3.3.4. Bioavailability study

Typical chromatograms obtained showing peaks for NVP at 1 h after administration of marketed suspension and in-house developed SEDDS F1 and F2 are shown in Figure 3.8(a), 3.8(b) and 3.8(c), respectively. The *in vivo* pharmacokinetic behavior of nevirapine with SEDDS and suspension were investigated. Figure 3.9 shows the plasma profiles of nevirapine after oral administration of the marketed suspension and developed SEDDS (F1 and F2).
Figure 3.8(a): Typical chromatogram obtained while analyzing plasma for NVP level after administration of the marketed suspension at 1 h.

Figure 3.8(b): Typical chromatogram obtained while analyzing plasma for NVP level after administration of the SEDDS F1 at 1 h.
Figure 3.8(c): Typical chromatogram obtained while analyzing plasma for NVP level after administration of the SEDDS F2 at 1 h.

Figure 3.9: Plasma concentration profile of nevirapine after oral administration of SEDDS and marketed suspension in rats (n = 4 and 20 mg/kg)
The pharmacokinetic parameters are given in Table 3.2. The $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow \infty}$ of the SEDDS were significantly higher than those of the suspension. However, there was not much difference with respect to $C_{\text{max}}$ for F1 and suspension; in fact the suspension showed slightly higher $C_{\text{max}}$ than that of F1. The SEDDS F1, having about 20% of oil has somewhat delay in action which may be due to the fact that drug is enclosed in the core and takes time to get released and absorbed into the intestinal linings. Suspension, initially, having drug particles with smaller diameter and some amount in dissolved form may be responsible for the initial rise in plasma concentration as seen in the plasma profile (Figure 3.7) and same can be an explanation for ex vivo intestinal permeation. $C_{\text{max}}$ of F2 was highest among all the three formulations evaluated. The developed SEDDS F1 consisted of less amount of oil (10%) compared to F2 is less prone to gastric emptying delays and resulted in faster absorption (Subramanian et al., 2004). The role of particle size in the performance of the formulation in vivo is generally less important than formulators have assumed. The main reason for this is that as soon as the dispersed formulation leaves the stomach it encounters the formidable digestive power of the small intestine. The fate of the drug after the formulation has been digested is a great deal more important than the initial particle size. Esters will be rapidly hydrolysed in the presence of pancreatic lipase and even the most commonly used surfactants (ethoxylated esters) are often rapidly hydrolysed. The physical state of the degradation products will be changed significantly by contact with the mixed bile salt micelles and the drug will partition between the various phases in the gut lumen, or could precipitate out if the total solvent capacity is reduced as a consequence of lipolysis.

The relative bioavailabilities of nevirapine SEDDS were approximately 1.92-fold and 1.98-fold for F1 and F2 formulations, respectively when compared with the marketed suspension. There was no statistical significance difference between F1 and F2 for $C_{\text{max}}$, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow \infty}$. Therefore, SEDDS might be a promising approach to the oral delivery of nevirapine.
Table 3.2: 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></th>
<th>MKT</th>
<th>SEDDS (F1)</th>
<th>SEDDS (F2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$AUC_{0-\infty}$ (ng.h/mL)</strong></td>
<td>7082.736 ± 1941.31</td>
<td>10419.63 ± 2134.416</td>
<td>13868.37 ± 1892.631</td>
</tr>
<tr>
<td><strong>$AUC_{0-\infty}$ (ng.h/mL)</strong></td>
<td>7422.146 ± 2100.749</td>
<td>14266.72 ± 1958.154</td>
<td>14729.68 ± 2294.147</td>
</tr>
<tr>
<td><strong>C$_{\text{max}}$ (ng/mL)</strong></td>
<td>1509.99 ± 517.602</td>
<td>1317.661 ± 569.047</td>
<td>3054.04 ± 1522.733</td>
</tr>
<tr>
<td><strong>T$_{\text{max}}$ (h)</strong></td>
<td>1.0 ± 0.0</td>
<td>1.5 ± 0.577</td>
<td>1.5 ± 0.577</td>
</tr>
<tr>
<td><strong>Relative bioavailability (%)</strong></td>
<td>---</td>
<td>192.218</td>
<td>198.455</td>
</tr>
</tbody>
</table>

3.3.5. Stability Studies

Chemical and physical stability of the nevirapine SEDDS were assessed at various storage conditions as per ICH guidelines. The developed formulations were stored in glass vials with rubber closure and aluminium-crimped tops. 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 3.4 and 3.5), when compared to initial characterization as shown in Table 3.3. No decline in the drug content was observed at the end of 3 months indicating that drug remained chemically stable in SEDDS.

Table 3.3: Initial characterization of nevirapine SEDDS (F1 and F2)

<table>
<thead>
<tr>
<th></th>
<th>F1</th>
<th>F2</th>
<th>F1</th>
<th>F2</th>
<th>F1</th>
<th>F2</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Droplet size D (nm)</strong></td>
<td>210.8</td>
<td>14.63</td>
<td>0.429</td>
<td>0.125</td>
<td>2.74</td>
<td>15.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Polydispersity Index (PdI)</strong></td>
<td>163.72</td>
<td>166.36</td>
<td>168.37</td>
<td>169.78</td>
<td>166.58</td>
<td>170.22</td>
<td>161.28</td>
<td>167.2</td>
</tr>
<tr>
<td><strong>Zeta potential (mV)</strong></td>
<td>101.78</td>
<td>101.24</td>
<td>99.83</td>
<td>103.19</td>
<td>101.07</td>
<td>99.50</td>
<td>100.15</td>
<td>100.15</td>
</tr>
</tbody>
</table>

Table 3.4: Stability studies of nevirapine SEDDS F1 at various storage conditions

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>25 ± 2°C (RT)</th>
<th>30 ± 2°C/65 ± 5% RH</th>
<th>40 ± 2°C/75 ± 5% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
<td>1 M</td>
<td>2 M</td>
<td>3 M</td>
</tr>
<tr>
<td><strong>Droplet Size D (nm)</strong></td>
<td>DW</td>
<td>209.8</td>
<td>214.29</td>
</tr>
<tr>
<td>0.1N HCl</td>
<td>225.35</td>
<td>218.38</td>
<td>220.54</td>
</tr>
<tr>
<td>PBS</td>
<td>163.72</td>
<td>166.36</td>
<td>168.37</td>
</tr>
<tr>
<td><strong>Polydispersity index (PdI)</strong></td>
<td>DW</td>
<td>0.421</td>
<td>0.372</td>
</tr>
<tr>
<td>0.1N HCl</td>
<td>0.427</td>
<td>0.442</td>
<td>0.338</td>
</tr>
<tr>
<td>PBS</td>
<td>0.395</td>
<td>0.359</td>
<td>0.331</td>
</tr>
<tr>
<td><strong>Zeta Potential (mV)</strong></td>
<td>DW</td>
<td>-4.3</td>
<td>-6.98</td>
</tr>
<tr>
<td>0.1N HCl</td>
<td>3.52</td>
<td>6.27</td>
<td>4.59</td>
</tr>
<tr>
<td>PBS</td>
<td>-3.46</td>
<td>-5.27</td>
<td>-4.71</td>
</tr>
<tr>
<td><strong>Drug Content (%)</strong></td>
<td>101.61</td>
<td>101.24</td>
<td>99.83</td>
</tr>
</tbody>
</table>
Table 3.5: Stability studies of nevirapine SEDDS F2 at various storage conditions

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>25 ± 2°C (RT)</th>
<th>30 ± 2°C/65 ± 5% RH</th>
<th>40 ± 2°C/75 ± 5% RH</th>
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<tbody>
<tr>
<td>Parameters ↓</td>
<td>Medium↓</td>
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<td>Droplet Size D (nm)</td>
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<td></td>
<td>0.1N HCl</td>
<td>15.03</td>
<td>15.39</td>
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<tr>
<td></td>
<td>PBS</td>
<td>14.27</td>
<td>12.79</td>
</tr>
<tr>
<td>Polydispersity index (PdI)</td>
<td>DW</td>
<td>0.168</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>0.201</td>
<td>0.327</td>
</tr>
<tr>
<td></td>
<td>PBS</td>
<td>0.321</td>
<td>0.294</td>
</tr>
<tr>
<td>Zeta Potential (mV)</td>
<td>DW</td>
<td>-4.62</td>
<td>-9.35</td>
</tr>
<tr>
<td></td>
<td>0.1N HCl</td>
<td>14.28</td>
<td>15.39</td>
</tr>
<tr>
<td>Drug Content (%)</td>
<td>100.53 ± 2.25</td>
<td>100.21 ± 3.19</td>
<td>101.29 ± 0.18</td>
</tr>
</tbody>
</table>

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

3.4. Conclusion

A SEDDS containing poorly water-soluble drug, nevirapine, was formulated for oral application. 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 formulations of the SEDDS consisted of 20% Caprylic acid, 64% mixture of Labrasol and Tween 80 (1:3) and 16% Transcutol P (F1) and 10% Caprylic acid, 77.14% mixture of Labrasol and Tween 80 (1:3) and 12.86% Transcutol P (F2). Both the SEDDS formulations had sufficient drug loading, rapid self-emulsification in aqueous media, and forming droplet not more than 260 nm. The in vivo study showed that there is no significant difference among both the developed formulations, F1 and F2, but showed greater extent of absorption than the marketed suspension. It is desirable to have less amount of surfactants and more amount of oil and therefore, the formulation F1 with 20% of Caprylic acid as oil, 64% mixture of Labrasol and Tween 80 (1:3) as surfactant and 16% Transcutol P as co-surfactant is acceptable. The relative bioavailabilities of SEDDS to suspension were 192.22 % and 198.45 % for F1 and F2, respectively. The data overall showed little apparent correlation between emulsion droplet size and oral absorption in rats, but differences in exposures were generally not large enough to be statistically significant. Both formulations did show significant differences in exposures, and these results were not consistent with a strong dependence on emulsion droplet size. However, there was an increase in absorption with increased in surfactant content. The increase in oral bioavailability could be a consequence of delivering the drug in a solubilised and rapidly dispersed manner. The results obtained illustrate the magnitude of bioavailability enhancement that can be achieved through rational design of lipid-based formulations.
REFERENCES


ICH harmonized tripartite guideline, Stability testing of new drug substances and products Q1A (R2).


SECTION II
Itraconazole
Page no. 116 to 124
II.1. Drug profile

**ITRACONAZOLE**

(Bennett, 2001; Beule, 1996; Merck Index, 2006; Moffat et al., 2004; Sweetman, 2005)

![Figure II.1. Structure of Itraconazole](image)

**Chemical Formula:** C\textsubscript{35}H\textsubscript{38}Cl\textsubscript{2}N\textsubscript{8}O\textsubscript{4}

**Molecular weight:** 705.64

**CAS name:** 2-butane-2-y1-4-[4-[4-[[2R,4S]-2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-y1]methoxy]phenyl]piperazine-1-yl]phenyl]-1,2,4-triazol-3-one

**CAS registry No.:** 84625-61-6

**Physicochemical properties:**

**Description:** Off-White Crystalline Solid

**Solubility:** Practically insoluble in water and dilute acidic solution; soluble in dichloromethane (DCM), sparingly soluble in tetrahydrofuran (THF).

**Dissociation constant (pKa):** 3.7

**Partition coefficient/Polarity:** Log P (n-octanol BUFFER pH 8.1), 5.66

**Melting point:** 166.2°C

**Mechanism of action:**

Itraconazole is a triazole antifungal drug which inhibits cytochrome P450-dependent enzyme, essential for ergosterol synthesis in fungal cell membranes. It has slightly wider spectrum of activity than ketoconazole various fungal species.
Pharmacokinetics

Absorption: Itraconazole is absorbed from the GIT when administered by orally either as a capsule containing drug coated onto sugar spheres or as an oral liquid formulated with hydroxypropyl-β-cyclodextrin. Absorption from the capsule formulation (Sporanox® Capsule) is enhanced by an acidic gastric environment and is greatest when doses are taken with food. Absorption from the oral liquid (Sporanox® Oral Solution) is not dependent on an acid environment, and absorption is greatest in the fasting state. Peak plasma concentrations are achieved between 1.5 and 5 hours after a dose of either formulation, and steady state is reached within 15 days during daily dosing. Peak plasma concentrations at steady state of about 2µg/mL have been reported following daily doses of 200 mg.

Distribution: Drug is highly protein bound; only 0.2% circulates as free drug. It is widely distributed but only small amounts diffuse into the CSF. Concentrations attained in the skin, sebum, pus, and many organs and tissues are several times higher than simultaneous plasma concentrations. Therapeutic concentrations of drug remain in the skin and mucous membranes for 1-4 weeks after the drug is discontinued. Small amounts are distributed into the breast milk. Volume of distribution 10.7 L/kg also reported as 561L.

Metabolism/Elimination: It is metabolised in the liver mainly by cytochrome P450 isoenzyme CYP3A4. The major metabolite hydroxyitraconazole, has antifungal activity comparable with that of itraconazole. The drug is also excreted as inactive metabolites in the bile or urine; 3-18% is excreted in the faeces as unchanged drug. Small amounts are eliminated in the stratum corneum and hair. Itraconazole is not removed by dialysis. The elimination half-life following a single 100 mg dose has been reported as 20 hours; the half-life increases to 30-40 hours with continued administration. The reported plasma clearance is about 18.7 to 22.9 L/h.

Interactions: Enzyme-inducing drugs such as carbamazepine, isoniazid, nevirapine, phenytoin, rifabutin or rifampicin may decrease plasma concentrations of itraconazole. Conversely, enzyme inhibitors such as clarithromycin, indinavir or ritonavir may increase plasma concentrations of itraconazole. Use of drugs that reduces stomach acidity, such as antimuscarinics, antacids, proton pump inhibitors and histamine H₂-receptor antagonists, may reduce the absorption of itraconazole.
Itraconazole may interfere with drugs metabolised by hepatic microsomal enzymes, especially the cytochrome P450 isoenzyme CYP3A4, hence the warnings that plasma concentrations of ciclosporin, cisapride, felodipine, midazolam, quinidine, sildenafil, sirolimus, atorvastatin, verapamil and warfarin may be increased. Concentrations of HIV-protease inhibitors such as indinavir, ritonavir, saquinavir, may also be increased. Other drugs that may be affected include alprazolam, buspirone, busulfan, diazepam, digoxin, oral hypoglycaemics and the vinca alkaloids. The efficacy of oral contraceptives might be reduced.

Adverse Reactions: The most common adverse effects include dyspepsia, abdominal pain, nausea, constipation, diarrhoea (with the oral liquid), headache, and dizziness. Others include allergic reactions such as pruritus, rash, urticaria and angiodema. Isolated cases of the Stevens-Johnson syndrome have been associated with itraconazole.

II.2. Background of the study

Introduction

Itraconazole or 2-butan-2-yl-4-[4-[4-[4-[[2R,4S]-2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-1,2,4-triazol-3-one, invented in 1984 by Janssen Pharmaceutica. Itraconazole is a broad spectrum antifungal compound developed for oral, parenteral and topical use and is disclosed in U.S. Patents (Berndl et al., 2009; Heeres and Backx, 1981).

Since the discovery of the highly potent antifungal itraconazole (Sporanox®, Janssen Pharmaceutica) in the late 1970s, numerous patent applications and issued patents have appeared that describe and claim improvements for methods, manufacturing processes, formulations, enantiomers and uses for this commercially successful antifungal agent. In at least one case, the improvements have led to a new formulation product with proven clinical benefits. The patent history of itraconazole is well reviewed by Hector (2003).

The drug was approved by the FDA on September 11, 1992, for use in the treatment of adults co-infected with *Penicillium marneffei* and HIV. It is also approved for use as an alternative agent for the treatment or suppressive maintenance of cryptococcal meningitis in patients with AIDS and other immunocompromised conditions. (AIDS info, http://www.aidsinfo.nih.gov/DrugsNew/DrugDetail). Itraconazole is an orally active triazole antimycotic agent, which is active against a broad spectrum of fungal species.
Section II

Itraconazole

including *Cryptococcus, Candida, Aspergillus, Blastomyces* and *Histoplasma capsulatum var. capsulatum* (Odds et al., 2000; Saag and Desmukes, 1988).

Itraconazole is a very poorly water soluble weak base (pKa = 3.7), possessing an aqueous solubility of approx. 1 ng/mL at neutral pH and approx. 4 µg/mL at pH 1. The n-octanol/water partition coefficient is 5.66 at a pH of 8.1 (Beule, 1996; Jung et al., 1999; Peeters et al., 2002) Despite the high lipophilicity, resulting in a high cell membrane permeability its poor aqueous solubility makes the development of a sufficiently bioavailable itraconazole formulation a challenge.

It is ionized only at a low pH, such as gastric juice. Therefore, on oral administration, the gastric acidity is needed for adequate dissolution. The bioavailability of itraconazole is known to be increased after a meal as compared with that found in the fasting state (Barone et al., 1993). Since the bioavailability of poorly water-soluble drugs can be influenced by interactions with food or by the physicochemical conditions in the gastrointestinal (GI) tract (Dongowski et al., 2005), oral preparation of itraconazole is commonly prescribed to be administered according to a fixed dosing schedule, especially, to be immediately taken after meals. The oral bioavailability is maximal when Sporanox® capsules are taken with a full meal (Physician’s Desk Reference, 2000).

Moreover, the solubility of some commercially available formulations containing itraconazole falls rapidly with a small increase of pH. The solubility difference between pH 1.6 and 2.4 reaches to 5.3 times. Thus, the pH-dependence of the commercial Formulations may cause inter- and intra-individual absorption variation. For example, as for AIDS patients having a higher pH in stomach because of their poor gastric secretion, absorption was reported to be no more than 50% of that of normal people. Therefore, it has been recommended to take the formulation with a drink like a cola that can decrease the pH of stomach. It has been recommended to take it after meal because the higher pH of stomach before meal may reduce its absorption (Jeon et al, 2009).

II.3. Scope and objectives of the present work

Many technological methods to improve the dissolution characteristics of poorly water-soluble drugs have been reported in literature (Leuner and Dressman, 2000), such as micronization, formation of solvates, adsorbates, complexes, microspheres, or more often, solid dispersions. In the case of itraconazole, a number of studies have been taken to improve its solubility, such as forming complexes with hydroxypropyl-β-cyclodextrin
(Miyake et al., 1999; Peeters et al., 2002), solid dispersions by spray drying method (Jung et al., 1999; Yoo et al., 2000), solid dispersion by hot melt-extrusion (Verreck et al., 2003), solid dispersion by supercritical fluid (Lee et al., 2005); preparation of amorphous itraconazole (Janssens et al., 2008); particle engineered compositions of itraconazole using supercritical carbon-dioxide (Hassan et al., 2004); nanosuspension (Nakarani et al., 2010); solid solution (Kapsi and Ayres, 2001) and use of 1-methyl-pyrrolidone as a solubilising agent (Uch et al., 1999). In spite of the efforts taken for enhancing the solubility and bioavailability of itraconazole, the issue still remains a challenge.

Several available patents also teach the preparation of pellets by fluidized-bed coating with water-soluble polymers (Gillis et al., 1997), or melt extrusion with water-soluble polymers (Baert et al., 2006); fused mixture (Woo et al., 2000); viscous glassy composition (Woo et al., 2005); oral solution using alcoholic solvent and acid (Sherman, 2007); deuterium enriched itraconazole (Czarnik, 2009); soluble crystalline form including salts, co-crystals and related solvates (Remenar et al., 2009); solid dispersion (Berndl et al., 2009; Jeon et al., 2009).

Oral Solution (Sporanox®) is forbidden to be used in patients with impaired renal function, not because of the toxicity of the drug itself, but the adjuvant hydroxypropyl β-cyclodextrin (HP-β-CD). Each milliliter of Oral Solution (Sporanox®) contains 10mg of itraconazole solubilised by 400mg of HP-β-CD as an inclusion complex. Following a single dose of 200mg Oral Solution (Sporanox®) to the subjects with severe renal impairment, clearance of HP-β-CD was six-fold reduced compared with subjects with normal renal function. Although its clinical relevance is unknown, it has been reported that HP-β-CD produces pancreatic adenocarcinoma in a rat carcinogenicity study (Physician Desk Reference, 2000).

Itraconazole is currently available as an oral formulation Sporanox® Itraconazole capsules. The capsules contain 100 mg of itraconazole coated on sugar spheres. These capsules are currently believed to contain residual levels of methylene chloride and original Sporanox® capsules were reformulated to the USP limit for methylene chloride which is 500 micrograms per day. Current Sporanox technology produces a product having approximately 60% less bioavailability under fasting conditions (Namburi et al., 2003). Some of the itraconazole formulations available in Indian market are shown in Table II.1.
Table II.1: Commercially available itraconazole formulations in India

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Dosage form</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporanox</td>
<td>Capsule</td>
<td>Jhonson &amp; Jhonson</td>
</tr>
<tr>
<td>Canditral</td>
<td>Capsule</td>
<td>Glenmark</td>
</tr>
<tr>
<td>Itaspor</td>
<td>Capsule</td>
<td>Intas</td>
</tr>
<tr>
<td>Flucover</td>
<td>Capsule</td>
<td>Saga Lab</td>
</tr>
</tbody>
</table>

The importance of using self-emulsifying drug delivery system for improving solubility and bioavailability of poorly water soluble drug, itraconazole has been carried out in the present research work. Solubility improvement and enhancement in gut absorption by formulating itraconazole in a self emulsifying formulation was the main objective of this research work.

The specific objectives were:

Exploration of self-emulsifying drug delivery system to improve the solubility and dissolution rate of poorly water soluble drug itraconazole. The solubilizing potential of Soluphor® P (2-pyrrolidone) and its suitability in developing a self emulsifying formulation was evaluated. The role of other GRAS lipid-based excipients in the improvement of solubility and in vivo absorption of drug was also carried out. The effect of ratios of surfactant:oil on droplet size, in vitro diffusion, ex vivo permeation and in vivo absorption was investigated. The in vivo performances of the developed formulations were compared with marketed capsule using a rat model.
REFERENCES


Section II

Itraconazole


