2. MATERIALS AND METHODS

2.1 Materials

Procurement of drug and excipients

Drugs, excipients, chemicals/ reagents and instruments used for various experiments are enlisted in Table 2.1 and 2.2.

Table 2.1: Drug, excipients, chemicals/ reagents used for various experiments

<table>
<thead>
<tr>
<th>Material</th>
<th>Gifted/Supplied by</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug</strong></td>
<td></td>
</tr>
<tr>
<td>Telmisartan (Abbreviation used further: TEL)</td>
<td>Glochem Industries Ltd. Vishakhapatnam, AP, India</td>
</tr>
<tr>
<td>Verapamil Hydrochloride (Abbreviation used further: VPH)</td>
<td>Piramal Healthcare Limited, Goregaon East, Mumbai, MS, India</td>
</tr>
<tr>
<td><strong>Oil</strong></td>
<td></td>
</tr>
<tr>
<td>Captex 355</td>
<td>ABITEC Corporation</td>
</tr>
<tr>
<td>Campul MCM</td>
<td></td>
</tr>
<tr>
<td>Acrysol EL 135</td>
<td>Corel Pharma, Gujarat, India</td>
</tr>
<tr>
<td>Capryol 90</td>
<td></td>
</tr>
<tr>
<td>Maisine 35-1</td>
<td>Gattefosse India Pvt. Ltd., Mumbai</td>
</tr>
<tr>
<td>Capryol 90</td>
<td></td>
</tr>
<tr>
<td>Arachis oil</td>
<td></td>
</tr>
<tr>
<td>Castor oil</td>
<td></td>
</tr>
<tr>
<td>Coconut oil</td>
<td>Loba Chemie Pvt. Ltd., Mumbai</td>
</tr>
<tr>
<td>Cotton seed oil</td>
<td></td>
</tr>
<tr>
<td>Ethyl oleate</td>
<td></td>
</tr>
<tr>
<td>Oleic acid</td>
<td></td>
</tr>
<tr>
<td>Terpentine oil</td>
<td></td>
</tr>
</tbody>
</table>
### Chapter 2 | Materials and Methods

#### Surfactant and co-surfactant

<table>
<thead>
<tr>
<th>Surfactant and co-surfactant</th>
<th>Make</th>
<th>Place</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cremophore RH 40</td>
<td>BASF, Mumbai</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Labrafil 2125 cs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labrasol</td>
<td>Gattefosse India Pvt. Ltd., Mumbai</td>
<td></td>
</tr>
<tr>
<td>Plurol oleique</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transcutol P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG 400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG 200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Span 20</td>
<td>Loba Chemie Pvt. Ltd., Mumbai</td>
<td></td>
</tr>
<tr>
<td>Span 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Solid carriers

<table>
<thead>
<tr>
<th>Solid carriers</th>
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<tbody>
<tr>
<td>HPMC K15 M</td>
<td>Agenta Pharma, Mumbai</td>
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</tr>
<tr>
<td>Maltodextrin</td>
<td>Loba Chemie Pvt. Ltd., Mumbai</td>
<td></td>
</tr>
<tr>
<td>Neusilin US2</td>
<td>Fuji chemicals Japan</td>
<td></td>
</tr>
<tr>
<td>Aerosil 200</td>
<td>S. D. Fine Chemicals, Mumbai</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Make</th>
<th>Place</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSC</td>
<td>SIIO 6300, Japan</td>
<td>Diya Labs, Mumbai, MS, India</td>
</tr>
<tr>
<td>PXRD</td>
<td>Bruker D2 Phaser</td>
<td>Shivaji University, Kolhapur, MS, India</td>
</tr>
<tr>
<td>FTIR</td>
<td>BRUKER ALPHA-E</td>
<td>Gaurishankar Education Society’s Satara College of Pharmacy, Satara, MS, India</td>
</tr>
<tr>
<td>Malvern Zetasizer</td>
<td>Malvern Instruments Nano ZS90</td>
<td>Indian Institute of Science, Bangalore, Karnataka, India</td>
</tr>
<tr>
<td>SEM</td>
<td>JEOL-JSM- 6360, Japan</td>
<td>Shivaji University, Kolhapur, MS, India</td>
</tr>
<tr>
<td>Digital Centrifuge</td>
<td>Remi Motors Ltd., Mumbai</td>
<td></td>
</tr>
</tbody>
</table>
Materials and Methods

Chapter 2

Dept. of Pharmaceutical Sciences, JJJ University, Rajasthan

Animals used

Table 2.3: Animals used in experiment

<table>
<thead>
<tr>
<th>Animal</th>
<th>Species / Common name</th>
<th>Weight</th>
<th>Gender</th>
<th>Proposed source of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>Rabbits (New Zealand, White)</td>
<td>1.6-2 Kg</td>
<td>Any Sex</td>
<td>Animal House of Tatyasaheb Kore College of Pharmacy, Warananagar, MS, India</td>
</tr>
<tr>
<td>Rat</td>
<td>Wistar rat</td>
<td>250-300 g</td>
<td>Male</td>
<td>Animal House of Tatyasaheb Kore College of Pharmacy, Warananagar, MS, India</td>
</tr>
</tbody>
</table>
2.2 Drug profile

2.2.1 Telmisartan (Neil M. J., et al., 2006 and Burnier M., 2009)

Chemical Formula  \( \text{C}_{33}\text{H}_{30}\text{F}_{2}\text{N}_{4}\text{O}_{2} \)

Molecular weight  514.617 g/mol

Chemical Structure

Category  Angiotensin II (AT1) receptor antagonist

Description  White or slightly yellowish, crystalline powder

Melting range  261 - 263 °C

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral bioavailability</td>
<td>42 %</td>
</tr>
<tr>
<td>Plasma half-life mean</td>
<td>24 h</td>
</tr>
<tr>
<td>Volume of distribution</td>
<td>7.0 L/Kg</td>
</tr>
<tr>
<td>Plasma protein binding</td>
<td>&gt; 99.5 %</td>
</tr>
<tr>
<td>Plasma clearance</td>
<td>&gt; 800 mL/min</td>
</tr>
<tr>
<td>Onset of action</td>
<td>Within 3 h after administration of a single dose</td>
</tr>
</tbody>
</table>
### 2.2.2 Verapamil hydrochloride (Indian Pharmacopoeia, 1996, The United State Pharmacopoeia 2000 and Tripathi K. D. 2003)

<table>
<thead>
<tr>
<th>Chemical Formula</th>
<th>C_{13}H_{12}F_{2}N_{6}O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>491.07 g/mol</td>
</tr>
<tr>
<td>Chemical Structure</td>
<td><img src="image" alt="Chemical Structure of Verapamil" /></td>
</tr>
<tr>
<td>Category</td>
<td>Calcium channel blocker</td>
</tr>
<tr>
<td>Description</td>
<td>White, crystalline powder</td>
</tr>
<tr>
<td>Melting range</td>
<td>140 - 144 °C</td>
</tr>
<tr>
<td>Pharmacokinetic parameters</td>
<td></td>
</tr>
<tr>
<td>Oral bioavailability</td>
<td>20 - 35 %</td>
</tr>
<tr>
<td>Plasma half-life mean</td>
<td>2.8 - 7.4 h</td>
</tr>
<tr>
<td>Volume of distribution</td>
<td>5.0 L/Kg</td>
</tr>
<tr>
<td>Plasma protein binding</td>
<td>90 %</td>
</tr>
<tr>
<td>Plasma clearance</td>
<td>0.9 L/h/kg</td>
</tr>
<tr>
<td>Onset of action</td>
<td>&lt; 1.5 minutes (i.v.), 30 minutes (oral)</td>
</tr>
<tr>
<td>Disposition</td>
<td>About 90% bound to plasma protein. 70% eliminated by kidney; 15% by gastrointestinal tract.</td>
</tr>
</tbody>
</table>

**Mechanism of action**

It dilates arterioles and has some a adrenergic blocking activity – decreases total peripheral resistance but BP is only modestly lowered. The heart rate generally decreases, A-V conduction is slowed, but cardiac output is maintained by
reflex sympathetic stimulation. Coronary flow is increased.

2.3 Excipient profile (Rowe R. C., et al., 2009)

2.3.1 Oleic acid

Chemical Name (Z)-9-Octadecenoic acid

CAS Registry Number 112-80-1

Empirical Formula C\textsubscript{18}H\textsubscript{34}O\textsubscript{2}

Molecular Weight 282.47

Structural Formula

![Structural Formula of Oleic Acid]

Functional Category Emulsifying agent; skin penetrant.

Description A yellowish to pale brown, oily liquid with a characteristic lard-like odor and taste.

Solubility Miscible with benzene, chloroform, ethanol (95%), ether, hexane, and fixed and volatile oils; practically insoluble in water.

Viscosity (dynamic) 26 mPa s (26 cP) at 25°C

Safety Oleic acid is used in oral and topical pharmaceutical formulations.
2.3.2 **Tween 80**

**Chemical Name** Polyoxyethylene 20 sorbitan monooleate

**CAS Registry Number** 9005-65-6

**Empirical Formula** $C_{64}H_{124}O_{26}$

**Molecular Weight** 1310

**Structural Formula**

![Structural formula of Tween 80]

**Functional Category** Dispersing agent; emulsifying agent; nonionic surfactant; solubilizing agent; suspending agent; wetting agent.

**Description** Polysorbates have a characteristic odor and a warm, somewhat bitter taste. Their colors and physical forms at 25°C.

**Viscosity (dynamic)** 425 mPa s at 25°C

**Regulatory Status** GRAS listed. Included in the FDA Inactive Ingredients Database.

---

2.3.3 **PEG 400**

**Chemical Name** $\alpha$-Hydro-o-hydroxypoly (oxy-1,2-ethanediyl)

**CAS Registry Number** 25322-68-3

**Empirical Formula** $C_{2n}H_{4n}+2O_n+1$

**Molecular Weight** 380-420
Solubility  Liquid polyethylene glycols are soluble in acetone, alcohols, benzene, glycerin, and glycols.

Viscosity  90.0 mm²/s (cSt) at 25°C

2.3.4 Castor oil

Chemical Name  Castor oil

CAS Registry Number  8001-79-4

Empirical Formula  C₁₈H₃₄O₃

Molecular Weight  298.4608

Functional Category  Emollient; oleaginous vehicle; solvent.

Viscosity (dynamic)  1000 mPa s (1000 cP) at 20°C
2.3.5 Labrasol

Functional Category  Dissolution enhancer; emulsifying agent; nonionic surfactant; penetration agent; solubilizing agent; sustained-release agent.

Description  It is roughly white oily liquid at room temperature. It has little odour and HLB value of 14. It possesses relative density of 1.06-1.07 at 20 °C.

Solubility  It is soluble in water and many organic solvent.

Regulatory Status  USP-NF/EP/ IIG

2.3.6 Transcutol P

Empirical Formula  \( \text{C}_6\text{H}_{14}\text{O}_3 \)

Molecular Weight  134.17356

Functional Category  A high purity solvent and solubilizer for poorly water soluble active pharmaceutical ingredients. Associated with improved drug penetration, permeation.

Description  Colourless liquid

Solubility  Soluble in water.

Safety  Safety of use is supported by extensive toxicological evaluations and precedence of use in approved pharmaceutical products.

Regulatory Status  USP-NF, EP, USIFA, IIG
2.3.7 Maltodextrin

Synonyms  PharmDry, Glucidex, Glucodry, Lycatab DSH, Maldex, Maltrin, Maltrin QD, Paselli MD10 PH, etc

Chemical Name  Maltodextrin

CAS Registry Number  9050-36-6

Empirical Formula  \((\text{C}_6\text{H}_{10}\text{O}_5)_n\cdot\text{H}_2\text{O}\)

Molecular Weight  900-9000

Structural Formula

Safety  Maltodextrin is a readily digestible carbohydrate with a nutritional value of approximately 17 kJ/g (4 kcal/g). As an excipient, maltodextrin is generally regarded as a nonirritant and nontoxic material.

2.3.8 HPMC K15 M

Synonyms  Hydroxy propyl methyl cellulose

CAS Registry Number  9004-62-0
Chapter 2  Materials and Methods

Empirical Formula  \((C_6H_{10}O_5)_n\cdot\text{H}_2\text{O}\)

Structural Formula

Viscosity (dynamic)  15000 cps

2.3.9 Neusilin US2

Synonyms  Aluminium magnesium silicate, Aluminosilicic acid (HAlSiO4), magnesium salt, Aluminum Magnesium Silicate, aluminum magnesium tetrakis(oxidanidyl)silane, aluminum magnesium tetraoxidosilane, Angast, Magnesium aluminate metasilicate, Magnesium aluminosilicate (MgAl2Si2O8), Magnesium aluminum silicate (MgAl2(SiO4)2), Neusilin, Neusilin FH 2, Neusilin FL 2, Neusilin UFL 2

Chemical Name  Aluminum magnesium tetraoxidosilane

CAS Registry Number  12511-31-8

Empirical Formula  \(\text{Al}_2\text{O}_3\cdot\text{MgO}\cdot1.7\text{SiO}_2\cdot\text{xH}_2\text{O}\)
Structural Formula

Functional Category  Functions for direct compression or wet granulation, oil adsorption capacity and flow enhancing properties.

Description  White amorphous granules and powder.

2.3.10 Aerosil 200

Chemical Name  Silica

CAS Registry Number  7631-86-9, 112945-52-5

Empirical Formula  SiO₂

Molecular Weight  60.08
2.4 Methods

2.4.1 Preformulation study


2.4.1.1 Characterization of TEL and VPH


TEL and VPH were tested for organoleptic properties such as appearance, colour, taste, etc.

*Melting Point determination*

Melting point determination of the obtained sample of TEL and VPH was done by open capillary method (Neil M. J., et al., 2006, Indian Pharmacopoeia, 2007, British Pharmacopoeia, 2009 and Vogel A. I., et al., 1978)

*pH*

pH of TEL and VPH was determined in a 5% w/v solution prepared with the aid of gentle heat. (Neil M. J., et al., 2006 and Indian Pharmacopoeia, 2007)

*Clarity and colour of solution*

Clarity and colour of TEL solution was determined by preparing solution of 0.5 g of TEL dissolved in 1M NaOH and diluted to 10 mL with same solvent. Clarity and colour of 5.0% w/v solution of VPH was determined in carbon dioxide-free water prepared with the aid of gentle heat. Clarity was determined manually. Results were compared with specifications given in Pharmacopoeia and certificate of analysis provided by drug sample provider. (Indian Pharmacopoeia, 2007 and British Pharmacopoeia, 2009)

*Loss on drying*

Loss on drying was determined by keeping 0.5 g of TEL and 1 g of VPH in oven at 105°C for 2 h. Results were compared with specifications given in Pharmacopoeia
and certificate of analysis provided by drug sample provider. (Indian Pharmacopoeia, 2007 and British Pharmacopoeia, 2009)

2.4.1.2 Spectroscopic study for TEL

A. UV Spectroscopy (Determination of $\lambda_{\text{max}}$):

**Preparation of Standard Curve** (Patel P. A., et al., 2010)

- **In methanol**

  The standard curve in methanol was prepared to analyze the drug content in different oils, surfactants and co-surfactants during solubility study of TEL. From this solution 10 mL was withdrawn and diluted to 100 mL with methanol. From this stock solution serial dilutions were made to obtain the solutions in concentration ranging from 2-18 $\mu$g/mL. The absorbance of the solution was measured at 296 nm.

- **In hydrochloric acid buffer pH 1.2**

  10 mg of TEL was dissolved in a small amount of methanol in 100 mL volumetric flask and the volume was made up to 100 mL using acid buffer pH 1.2 to obtain a concentration of 100 $\mu$g/mL. From this solution 10 mL was withdrawn and diluted to 100 mL with acid buffer pH 1.2. From this stock solution serial dilutions were made to obtain the solutions in concentration ranging from 2-18 $\mu$g/mL. The absorbance of the solution was measured at 296 nm.

- **In phosphate buffer pH 7.5**

  From this solution 10 mL was withdrawn and diluted to 100 mL with phosphate buffer pH 7.5. From this stock solution serial dilutions were made to obtain the solutions in concentration ranging from 2-18 $\mu$g/mL. The absorbance of the solution was measured at 296 nm.

B. IR spectrum interpretation

The infrared absorption spectrum of pure TEL was recorded on FT-IR spectrophotometer (BRUKER ALPHA-E) and the spectrum analysis was done for functional groups. (Vogel A. I., et al., 1978 and Skoog D. A., et al., 2003)
2.4.1.3 Spectroscopic study for VPH

A. UV Spectroscopy (Determination of $\lambda_{\text{max}}$):

The stock solution of VPH was prepared by dissolving accurately 10 mg of VPH in methanol in a 100 mL volumetric flask to obtain a concentration of 100 $\mu$g/mL. The UV spectrum was recorded in the range of 220-360 nm on Shimadzu UV-visible spectrophotometer (Shimadzu-1800, Japan) at 1 cm, slit width. (Skoog D. A., et al., 2003)

B. IR spectrum interpretation

The infrared absorption spectrum of pure VPH was recorded on FT-IR spectrophotometer (BRUKER ALPHA-E) and the spectrum analysis was done for functional groups. (Indian Pharmacopoeia, 2007, Vogel A. I., et al., 1978 and Skoog D. A., et al., 2003)

2.4.2 Determination of the solubility of drugs in oils, surfactants and co-surfactants

Different oils, surfactants, co-surfactants used to study solubility of TEL and VPH are mentioned in Table 2.4. 2 mL of different oils, surfactant, and co-surfactants were taken separately in small vial and excess amount of the drug was added to each vial. The vials were tightly closed and were stirred continuously for 72 h using mechanical shaker at 25°C. Then oils, surfactant, and co-surfactants were centrifuged at 10000 rpm for 10 min to separate undissolved drug. The supernatant was taken and diluted with methanol and solubility was quantified with UV-spectroscopy (Shimadzu 1800) at 296 nm for TEL and at 278 nm for VPH. (Yuan Y., et al., 2006, Hong J. Y., et al., 2006 and Balakrishnan P., et al., 2009)

Table 2.4: Oils, surfactants and co-surfactants used to determine solubility of TEL and VPH

<table>
<thead>
<tr>
<th>Oil</th>
<th>Surfactant and Co-surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrysol EL 135</td>
<td>Campul MCM</td>
</tr>
<tr>
<td>Arachis oil</td>
<td>Capryol 90</td>
</tr>
<tr>
<td>Capryol 90</td>
<td>Cremophore RH 40</td>
</tr>
</tbody>
</table>
Captex 355        Glycerol
Castor oil        Labrafac
Coconut oil       Labrafil 2125 cs
Cotton seed oil   Labrasol
Ethyl oleate      PEG 400
Maisine 35-1      PEG 200
Oleic acid        Plurol oleique
Terpentine oil    Span 20
                   Span 80
                   Transcutol P
                   Tween 20
                   Tween 80

2.4.3 Screening of surfactant and co-surfactant

Depending upon the hydrophobic lipophilic balance (HLB) system for surface active agents, the selection of surfactants for micro emulsion was done. The HLB value for each component (single and in combination) was determined wherever necessary. The surfactant in combination with co-surfactants was tried for screening a stable micro emulsion system which could incorporate the optimum amount of internal phase.

2.4.3.1 Screening of co-surfactants

Various co-surfactants (from solubility study of TEL: PEG 400 and PEG 200 and from solubility study of VPH: Transcutol P and Plurol oleique) were screened for SMEDDS formulation. Mixtures of 100 mg of co-surfactant, 200 mg of selected surfactant (Tween 80 for TEL and Labrasol for VPH) and 300 mg of selected oil phase were prepared and evaluated in the same manner as described in the above section for surfactant screening. (Date A. A., et al., 2007)

2.4.4 Construction of pseudo ternary phase diagram

2.4.4.1 Construction of pseudo ternary phase diagram for TEL liquid SMEDDS

From solubility study and screening of surfactants and co-surfactants, co-surfactant respectively and for preparation of stable SMEDDS, micro emulsion region was
identified by constructing pseudo ternary phase diagram containing different proportion of surfactant: co-surfactant i.e. S/Co (Km value 1:1, 2:1, 3:1, 1:2 and 1:3), oil and water. In brief Smix and oil were mixed at ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 in pre-weighed test tube.

2.4.4.2 Construction of pseudo ternary phase diagram for VPH liquid SMEDDS

From solubility study and screening of surfactants and co-surfactants, Castor oil, Labrasol and Transcutol P were selected as oil, surfactant and co-surfactant respectively and pseudo ternary phase diagram containing different proportion of surfactant: co-surfactant i.e. S/Co (Km value 1:1, 2:1, 3:1, 1:2 and 1:3), oil and water was constructed by adopting method described above.

2.4.5 Formulation of Liquid SMEDDS

2.4.5.1 Formulation of Liquid SMEDDS of TEL

From constructed phase diagrams Km value at which high micro emulsion region obtained was selected for further studies. The three formulations as TLM1, TLM2, and TLM3 were selected from this micro emulsion region and used for further evaluation studies. The % content of water, oil and Surfactant/co-surfactant in each selected formulation was determined. Composition of liquid SMEDDS of TEL is shown in Table 3.23.

2.4.5.2 Formulation of Liquid SMEDDS of VPH

From constructed phase diagrams Km value at which high micro emulsion region obtained was selected for further studies. The three formulations as VLM1, VLM2, and VLM3 were selected from this micro emulsion region and used for further evaluation studies. The % content of water, oil and Surfactant/co-surfactant in each selected formulation was determined. Composition of liquid SMEDDS of VPH is shown in Table 3.24.
2.4.6 Evaluation of Liquid SMEDDS

2.4.6.1 Evaluation of Liquid SMEDDS of TEL

*Thermodynamic stability studies*

Thermodynamic stability study of prepared SMEDDS was determined by carrying heating cooling cycle, centrifugation test and freeze thaw cycle. (Shafiq S., et al., 2007)

- **Heating cooling cycle**
- **Centrifugation test**
- **Freeze thaw cycle**

*Robustness to dilution*

It was studied by diluting liquid SMEDDS to 50, 100 and 1000 times with water, buffer pH 1.2, buffer pH 7.5 (for TEL SMEDDS) and buffer pH 6.8 (for VPH SMEDDS). (Date A. A., et al., 2007)

*Assessment of Efficiency of self-emulsification*

Efficiency of self-emulsification was assessed by procedure used by Khoo Shui-Mei et.al. (1998). The *in-vitro* performance of the formulations was visually assessed using the following grading system: (Khoo S. M., et al., 1998 and Shafiq S., et al., 2007)

*% Transmittance*

1 mL of Liquid SMEDDS was diluted to 100 mL distilled water and observed at 650 nm using UV–Vis spectrophotometer (Shimadzu-1800, Japan) (Date A. A., et al., 2007)
Globule size, PDI and Zeta potential

Dynamic Light Scattering

Its principle is that fine particles and molecules that are in constant random thermal motion, called Brownian motion, diffuse at a speed related to their size, smaller particles diffusing faster than larger particles. The speed of Brownian motion is also determined by the temperature, therefore precision temperature control is essential for accurate size measurement.

To measure the diffusion speed, the speckle pattern produced by illuminating the particles with a laser is observed. The scattering intensity at a specific angle will fluctuate with time, and this is detected using a sensitive avalanche photodiode detector (APD). The intensity changes are analysed with a digital autocorrelator which generates a correlation function.

To produce high quality data, the Zetasizer Nano series is designed to provide optimised components at every stage in the measurement chain from the laser and temperature control, through to the optical design and detector. (Zetasizer Nano series)
Figure 2.2: Schematics of Dynamic Light scattering instrument

Liquid SMEDDS was diluted to 10 times with distilled water and globule size, PDI and zeta potential were determined using Malvern Zetasizer (Nano ZS90). (Attwood D., 1994, Khoo S. M., et al., 1998, Shafiq S., et al., 2007 and Date A. A., et al., 2007)

**Viscosity**

The viscosity of the formulations (0.5 g) was determined as such without dilution using Brookfield LVDV II + pro viscometer using spindle S18 at 20 rpm at room temperature. (Shafiq S., et al., 2007)

### 2.4.6.2 Evaluation of Liquid SMEDDS of VPH

Liquid SMEDDS of VPH are also evaluated for thermodynamic stability studies, % transmittance, globule size, PDI and zeta potential, viscosity, dye solubilization test and cloud point measurement by adopting same procedure as that of for evaluation of liquid SMEDDS of TEL.

### 2.4.7 Formulation of S-SMEDDS

#### 2.4.7.1 Formulation of S-SMEDDS of TEL

S-SMEDDS of TEL was prepared by spray drying and adsorption technique.
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*Formulation of S-SMEDDS of TEL by spray drying technique*

Composition for formulation of S-SMEDDS of TEL by spray drying technique is shown in Table 2.5. Maltodextrin was dissolved in 100 mL distilled water. The liquid SMEDDS was added in above solution of maltodextrin. This emulsion was spray dried using lab spray dryer (JISL, LSD-48) under optimized conditions specified in Table 2.6. (Dollo G., et al., 2003)

**Table 2.5: Composition of S-SMEDDS of TEL by spray drying technique**

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Liquid SMEDDS</th>
<th>Maltodextrin</th>
<th>Water (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSM1</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>TSM2</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>TSM3</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2.6: Optimized conditions for spray drying for TEL S-SMEDDS**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Optimized values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet temperature</td>
<td>120 °C</td>
</tr>
<tr>
<td>Outlet temperature</td>
<td>70 °C</td>
</tr>
<tr>
<td>Aspirator speed</td>
<td>80 %</td>
</tr>
<tr>
<td>Feed rate</td>
<td>10 %</td>
</tr>
</tbody>
</table>

The prepared S-SMEDDS was collected from collector and cyclone separator and stored in sealed vial. These vials were kept in desiccators until analysis.
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Formulation of S-SMEDDS of TEL by adsorption technique

Composition for formulation of S-SMEDDS of TEL by adsorption technique is shown in Table 2.7. S-SMEDDS was prepared by mixing liquid SMEDDS containing TEL with Neusilin US2 and Aerosil 200 in 1:1 proportion.

Table 2.7: Composition of S-SMEDDS of TEL by adsorption technique

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Component (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neusilin US2</td>
</tr>
<tr>
<td>TSM4</td>
<td>10</td>
</tr>
<tr>
<td>TSM5</td>
<td>10</td>
</tr>
<tr>
<td>TSM6</td>
<td>10</td>
</tr>
<tr>
<td>TSM7</td>
<td>-</td>
</tr>
<tr>
<td>TSM8</td>
<td>-</td>
</tr>
<tr>
<td>TSM9</td>
<td>-</td>
</tr>
</tbody>
</table>

2.4.7.2 Formulation of S-SMEDDS of VPH

S-SMEDDS of VPH was formulated by adopting same procedure as that of for TEL. Here maltodextrin was replaced by HPMC K15M for getting sustained release of VPH. Composition for formulation of S-SMEDDS of VPH by spray drying technique is shown in Table 2.8. Optimized conditions for spray drying are shown in table 2.9.
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Table 2.8: Composition of S-SMEDDS of VPH by spray drying technique

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Liquid SMEDDS (g)</th>
<th>HPMC K15M (g)</th>
<th>Water (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSM1</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>VSM2</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>VSM3</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2.9: Optimized conditions for spray drying for VPH S-SMEDDS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Optimized values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet temperature</td>
<td>150 °C</td>
</tr>
<tr>
<td>Outlet temperature</td>
<td>70 °C</td>
</tr>
<tr>
<td>Aspirator speed</td>
<td>85 %</td>
</tr>
<tr>
<td>Feed rate</td>
<td>10 %</td>
</tr>
</tbody>
</table>

2.4.8 Characterization of S-SMEDDS

2.4.8.1 Micromeritic properties of S-SMEDDS and drug content

*Organoleptic properties*

The organoleptic properties of S-SMEDDS, like color, odor and physical appearance were checked by visual observation.

*Angle of repose*

*Bulk density*

*Compressibility Index*

*Hausner ratio*
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Spray drying process yield

Spray drying process yield was calculated for formulation TSM1, TSM2, TSM3, VSM1, VSM2 and VSM3 batches. (Hansen T., et al., 2004) The powder obtained from spray drying was mixed well and the product yield was calculated from the following formula:

\[ \text{Process yield} \ (\%) = \frac{W}{W_0} \]

Where \( W \) is Weight of product obtained from spray dryer and \( W_0 \) is Weight of total dissolved solid in micro emulsion.

Drug content

- **For S-SMEDDS obtained from spray drying technique**

Amount of drug present in S-SMEDDS obtained from spray drying technique (TSM1, TSM2, TSM3, VSM1, VSM2 and VSM3) was determined by taking 100 mg of S-SMEDDS and carrying out assay method for TEL and VPH as follows: The powder of each formulation was taken in the 100 mL volumetric flask. To this sufficient quantity of acid buffer pH 1.2 was added and the flask was shaken for 10 min. Then the volume was made with acid buffer pH 1.2. This was then filtered through nylon filter paper (0.45 µm), and from filtrate exactly 1 mL was transferred to another volumetric flask of 100 mL and volume was made with acid buffer pH 1.2. The UV absorbance of this was taken at 296 nm for TEL and at 278 for VPH. Drug content was then calculated by using calibration curve of plain TEL and VPH in acid buffer pH 1.2. (Indian Pharmacopoeia, 1996)

- **For S-SMEDDS obtained from adsorption technique**

Drug content was estimated by extracting TEL from S-SMEDDS. (Patel P. A., et al., 2010)
2.4.8.2 *In-vitro* dissolution studies

**In-vitro dissolution studies for S-SMEDDS of TEL**

The *in-vitro* dissolution study of S-SMEDDS of TEL and plain TEL were carried out using USP- type-II dissolution test apparatus. Hard gelatin capsule of size ‘0’ was filled with S-SMEDDS (equivalent to 20 mg of TEL) and plain TEL and put into each of 900 mL pH 1.2 and pH 7.5 buffer solutions at 37±0.5°C with 50 rpm rotating speed.

**In-vitro dissolution studies for S-SMEDDS of VPH**

Hard gelatin capsule of size ‘000’ was filled with S-SMEDDS (equivalent to 120 mg of VPH) and VPH:HPMC K15M physical mixture (PM). The *in-vitro* dissolution study of S-SMEDDS of VPH and PM were carried out using USP- type-I dissolution test apparatus at 37°C ± 0.5°C and 100 rpm speed. The dissolution studies were carried out in acid buffer of pH 1.2 for one hour and in phosphate buffer pH 6.8 for further seven hours under sink condition.

2.4.9 Selection of optimized formulations for further study

The S-SMEDDS batches of TEL and VPH were optimized on the basis of drug content, *in-vitro* drug release, globule size, PDI, zeta potential of reconstituted S-SMEDDS, spray dried process yield, etc and selected formulations were further studied for solid state characterization, morphological analysis, *ex-vivo* intestinal permeability studies, bioavailability and stability study.

2.4.10 Solid state characterization of S-SMEDDS

2.4.10.1 FTIR study

FTIR studies were done to assess whether any possible interaction among drug, maltodextrin HPMC K15M and Neusilin US2. Infrared spectrums of plain drug, physical mixture of drug and carriers and selected formulations of S-SMEDDS were recorded. From the spectrum analysis the compatibility of ingredients in the formulations was found out. (Raja R. K., et al., 2011)
2.4.10.2 Powder X-ray diffraction (PXRD)

Figure 2.3: X-ray powder diffractometer (D2 Phaser, Bruker AXS)

Figure 2.4: Schematic diagram of X ray diffractometer

To verify the physical state of TEL and VPH in pure state and the changes in the crystallinity in S-SMEDDS. PXRD of plain TEL, VPH, and selected S-SMEDDS was carried out using X-ray diffractometer (D2 Phaser, Bruker AXS). (Yi T., et al., 2008)
2.4.10.3 Differential scanning calorimetry (DSC)

Physical state of TEL and VPH in S-SMEDDS was characterized using differential scanning calorimeter. Thermograms of TEL, VPH, Maltodextrin, HPMC K15M, Neusilin US2 and S-SMEDDS were obtained using differential scanning calorimeter (SIIO 6300, Japan). (Yi T., et al., 2008)

2.4.11 Morphological analysis of S-SMEDDS

Morphological analysis of S-SMEDDS was done using SEM.
Scanning electron micrographs for VPH, TEL, Neusilin US2 and selected S-SMEDDS was taken using Scanning electron microscope (JEOL, Japan) at accelerating voltage at 3-5 kV to study surface topography. (Yi T., et al., 2008)

2.4.12 Ex-vivo intestinal permeability studies of TEL S-SMEDDS

All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee (Ref. No. IAEC/TKCP/2012/09) and all experiments were conducted as per the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Male Wister rat (250-300g) was sacrificed by CO₂ inhalation method. Intestine was isolated and cleaned properly. Reconstituted solution of S-SMEDDS and plain drug suspension was filled into the intestine which was tied at both the end. The tissue was placed in an organ bath with continuous aeration at 37°C. The receptor compartment (organ tube) was filled with phosphate-buffered saline pH 7.4 with 1% sodium lauryl sulphate. At predetermined time intervals, samples were withdrawn from the receptor compartment. Fresh buffer was used to replenish the receptor compartment. The samples were analyzed spectrophotometrically at 296 nm for the content of TEL. The percent diffusion was calculated using PCP-Disso v2.08 software and plotted against time. (Thakkar H., et al., 2011)

2.4.13 Bioavailability study of S-SMEDDS

2.4.13.1 Bioavailability study of S-SMEDDS of TEL

High Pressure Liquid Chromatography

Instrumental specification: Sample was resolved on a Agilent TC C18 (150mm X 4.6 mm i.d., particle size 5µ) column. HPLC - AGILLETNT-Model NO.1120 LC Compact System Consisting of Agilent TCC18 ODS column output signal was monitored and integrated using Lab monitor diagnosis and EZ-chrome.

Mobile Phase: Acetonitrile: Buffer (PH 3) [60:40]

Preparation of serum samples for calibration curve: 0.2 mL of spiked standard Wistar rat serum sample were vortexed (extracted) with acetonitrile using 1 mL v/v for 2 min and centrifuged for 5 min. After centrifugation supernatant layer up to 0.9
mL was separated and evaporated. Finally dry residue was reconstituted with mobile phase.

**Calibration curve range:** 1, 10, 20, 30, 50, 60 and 70 μg/mL in wistar rat serum

**Bioavailability in male Wistar rats**

The experimental procedures were approved by the Institutional Animal ethical committee Tatyasaheb Kore College of Pharmacy, Warananagar. (Ref. No. IAEC/TKCP/2012/09)

3 groups of rats were used for the experiments each group consists 3 rats. First group was administered aqueous suspension of TEL. Second group was administered with aqueous solution of TEL S-SMEDDS (equivalent to 20 mg of TEL. Third group was kept as controlled. (Kim J. Y., et al., 2000 and Setthacheewakul S., et al., 2010)

The samples were centrifuged at 13,000 rpm 20 ºC for 10 min and plasma was separated. 0.2 mL of plasma was vortexed (extracted) with acetonitrile using 1mL v/v up to 2 min and centrifuged at 3000 rpm up to 5 min. From this centrifuged solution supernatant layer up to 0.9 mL was separated and evaporated to dryness. (Kumar G. V., et al., 2011)

**Biopharmaceutical evaluation** (Brahmankar D. M., et al., 1995)

The elimination rate constant (K) was estimated from the terminal slope of the plasma concentration-time curve values.

\[ K = C7 - C8/T8 - T7 \]

Elimination half-life \( t_{1/2} \) was calculated from quotient 0.693/K

\[ t_{1/2} = 0.693/K \]

Area under the plasma drug concentration- time curve up to measurable concentration, AUC\(_{(0-t)}\) was calculated using the trapezoidal rule. The area under the curve zero to infinity, AUC\(_{(0-\infty)}\) was calculated by equation:

\[ AUC_{(0-\infty)} = AUC_{(0-t)} + Ct/K \]
Where, \( C_t \) is the last measurable concentration.

To assess the degree of retardation of drug release mean residence time (MRT) was calculated.

\[
MRT = \frac{1}{K}
\]

Relative bioavailability (\( F_{rel} \)) of TEL from TSM1 and TSM4 to that of plain TEL suspension was calculated using formula: (Zhang Y., et al., 2012)

\[
F_{rel} = \left( \frac{AUC_{test} / Dose_{test}}{AUC_{reference} / Dose_{reference}} \right) \times 100
\]

2.4.13.2 Bioavailability study of S-SMEDDS of VPH

**High Pressure Liquid Chromatography**

**Instrumental specification:** Sample was resolved on a Agilent TC C18 (150mm X 4.6 mm i.d., particle size 5µ) column. HPLC - AGILLENT-Model NO.1120 LC Compact System Consisting of Agilent TCC18 ODS column output signal was monitored and integrated using Lab monitor diagnosis and EZ-chrome.

**Mobile Phase:** Acetonitrile: Acetate Buffer (PH 7) [65:35]

**Stock and Working standard solutions:** The stock solution of the VPH was prepared by dissolving 25 mg of pure drug in 100 mL volumetric flasks containing 50 mL of methanol, filtered and sonicated for about 15 min.

**Preparation of serum samples for calibration curve:** 0.2 mL of rabbit serum samples was vortexed (extracted) with acetonitrile using 1mL v/v for 2 min and centrifuged up to 5min. After centrifugation supernatant layer up to 0.9 mL was separated and evaporated. Finally dry residue was reconstituted with mobile phase.

**Calibration curve range:** 1, 10, 20, 30, 50, 60 and 70 µg/mL in wistar rat serum

**Bioavailability studies in Rabbits**

The Institutional Animal Ethical Committee approved the protocol for this study (Ref. No. IAEC/TKCP/2012/09). Blood samples were collected from marginal ear vein at
time intervals of 0, 1, 2, 3.5, 5, 8 and 12 h and analyzed using HPLC. (Mallick S., et al., 2000)

**Biopharmaceutical evaluation**

Different pharmacokinetic parameters were determined same as above.

### 2.4.14 Accelerated stability study

The hard gelatin capsules size ‘0’ filled with the S-SMEDDS of TEL (TSM1 and TSM4) and hard gelatin capsules size ‘000’ filled with the S-SMEDDS of VPH (VSM1) were stored in air-tight glass containers and protected from light. Samples maintained in a stability chamber under intermediate conditions and evaluated under accelerated conditions (45 °C ± 2 °C, 75 ± 5% RH) with humidity and temperature control, were taken at 0, 1, 2 and, 3 month for both the conditions. Appearance, self-emulsifying properties, and drug content of both optimized formulations of S-SMEDDS were evaluated. (Setthacheewakul S., et al., 2010)