CHAPTER-5
FORMULATION AND EVALUATION OF SOLID DISPERSION OF ATOVAQUONE

5.1 INTRODUCTION

The largest part drugs are commonly administered by oral route because of patient compliance and its convenience. To swallow a dosage forms is easy and a common way of taking medicine. Hence, compliance of patient and drugs treatments are classically more efficient with orally given medication in comparison with other drug administration routes. (Youn YS et al., 2006).

Poor solubility is a problem with excess of 90 % of drugs. It is anticipated that 40% of active pharmaceutical ingredients identified in combinatorial screening program engaged by many pharma companies have poor water solubility (Ohara T et al., 2005). Upon administration of a drug by oral route it initially dissolve in stomach and or intestine fluid and then diffuses through GI tract membrane to reach systemic-circulation. Hence, a poor aqueous soluble drug will naturally demonstrate dissolution-rate limited absorption, and drugs with less membrane permeabilities will usually show permeation-rate limited absorption. Therefore, the main focus areas of pharma research are to improve the oral bio-availability of drugs which includes (Lewis K et al., 2009).

The improvement of peroral bio-availability of poor water soluble drug is one of the main challenging parts of drug-development. Thus, particle size reduction, solubilization and salt formation have frequently been used to improve dissolution-rate and thus bioavailability and peroral absorption of such drug, but there are practical restrictions of such methods. The salt-formation is not possible for neutral compound and the synthesis of suitable salt form of drug that is weak acidic or weak basic may repeatedly not be feasible. These salt forms can be prepared; an improved dissolution-rate in the GI tract cannot be attained in lots of cases due to the reconversion of salt into aggregate of their particular acid or base derivatives. The solubilization of drug in organic media or in aqueous solvent by using surface active agents and co-solvents
leading to liquid formulation that is generally unwanted from the viewpoint of acceptability by patient and commercialization. Thus reduction in particle size is frequently used to enhance dissolution-rate, but there are practical limitations to how much reduction in size can be attained by such generally used techniques as grinding and controlled-crystallization. The use of superfine powder in a drug delivery system can also be awkward because of poor wettability and handling difficulties. (R. N. Sonpal et al., 2011).

Studies shows that drug in solid-dispersion need not essentially exist in the micronized-state. Part of the drug may be molecularly dispersed in matrices, thus forms a solid-dispersion (.Goldberg AH et al., 2006). When the solid dispersions are exposing to water, carriers dissolve and the drug releases as fine particles. Thus resultant improved surface area turn outs high dissolution-rate and bio-availability of poor water soluble drug. Thus in solid dispersion a part of drug dissolve instantly to saturate the GI fluids and excess drug precipitate as fine particle or oily globule of micron size.

5.2 Plan of Work and Methodology:
1. Literature survey:

Literature survey of Drug profile International Journals and from different journals.

2. Characterization and Identification of the Selected Drug:

Characterization and Identification will be carried out by official monographs given in Indian Pharmacopeia.

3. Preformulation Study:

Preformulation study will be carried out for excipient and active Pharmaceutical Ingredient.

4. Preparation of solid dispersion formulations.

Using various the methods and changing in the concentration of different Polymers.

5. Optimization of the Formulation:

The prepared formulation were tested using different evaluation parameter like drug release Profile, micro particles size and shape drug content and stability.

6. Characterization of optimized batch

The formulations were tested for
- Physicochemical parameters
- Drug and excipient interaction study.
- Determination of solubility
- Determination of dissolution rate

7. Stability Study

Accelerated Optimized batch carried out will be as per ICH Guideline.
5.3. Atovaquone- Drug Profile
Atovaquone is the anti-protozoal agent.

**Structural formula:**

![Molecular structure of Atovaquone](image)

**Figure 52: Molecular structure of Atovaquone**

**IUPAC name:**
trans -2-[4- (4-chlorophenyl) cyclohexyl]- 3-hydroxy-1,4- naphthalenedione

**Chemical formula:** \( C_{22}H_{19}ClO_3 \)

**Average molecular weight:** 366.837 g/mol

**State:** solid in nature.

**Storage:** Store in a cool, dry place and away from sunlight.

**Mechanism of Action:**
Atovaquone possesses a novel mode of action against *Plasmodium falciparum* by inhibition of the electron-transport system at the cytochrome bc-1 complex level. The Atovaquone also causes disintegration of the parasite mitochondria membrane likely in *Plasmodium falciparum*. Mechanism of action against *Plasmodium. carinii* is unknown. The synergistic activity between proguanil and atovaquone appears to be related to proguanil *per se* and metabolism to cycloguanil is not required.

**Mechanism of Resistance:**
Atovaquone-resistant *Plasmodium falciparum* parasites contain point mutation in the cytochrome-b genes.

**Pharmacokinetics:**

It is a high lipophillic substance with poor water solubility and limited oral bio-availability that varies with dose and diet. It is extensively bound to protein (>99.9%). The elimination half-lives in fasting healthy subjects given single oral doses of 225mg to 750mg was 70 to 84 hours.

**Treatments and prophylaxis of *Pneumocystis carinii* Pneumonitis.**

The recommended dose is 1500mg per day taken in two divided doses for 21 days for treatment and as a single dose of 1500mg for preventing taken with food.

**Adverse Effects:**

No serious or life-threatening adverse effects have been reported in individuals receiving Atovaquone.

**Pregnancy:**

In the absence of adequate and well-controlled study in pregnant woman Atovaquone only and in blend with proguanill must be given only in pregnancy. if the potential benefits overshadow the potential danger to the baby.

**Drug Interactions:**

Reduction in plasma concentrations of Atovaquone are seen when given in combination with Rifampin, Rifabutin and Metaclopramide. Atovaquone increases the AUC of zidovudine through inhibition of Glucuronidation.
5.4 Poly Vinyl Pyrrolidone-Profile (Raymond et al., 2006)

Structural formula:

![Figure 53: Molecular structure of PVP](image)

Nonproprietary names:
- USP: povidone
- BP: povidone
- Ph Eur: polyvidonum
- JP: povidone

Chemical name: R1-ethenyl-2-pyrrolidinone homopolymer.

Synonym:
- E-1201: kollidion; plasodone; poly[1- (2- oxo- 1-pyrrolidinyl) ethylene ]; polyvinyl pyrrolidone; Polyvidone;1-vinyl 2-pyrrolidinone polymers, PVP.

Empirical formula: \((\text{C}_6\text{H}_9\text{NO})_n\).

Molecular weight: 2500-3000000.

Category:
- Suspending agent, disintegrating agent, binding agent in tablets, the dissolution aid.

Description:
Povidone is a fine, creamy-white, and almost smell less, hygroscopic in nature. Povidone with K– value equal or less than 30.00 are prepared by spray drying and exist as sphere. Povidone with K90 and high K-values. povidone is prepared by drum-drying and exist as plate.

**Typical parameter:**

- Density (B): 0.409g/cm³.
- Density (T): 0.508g/cm³.
- Density (true): 1.180g/cm³.
- Flow ability: 20g/s for povidone k-15.
  16g/s for povidone k-29/32.
- Melting point: softens at 150°C.

**Moisture content:** It is highly hygroscopic having larger amount of moisture absorbed at lower relative humilities.

**Particle size:**

Kolidon 25/30 :90 % >50um, 50 % >100um, 5 % > 200um;

kolidon 90:90 % >200um, 95 % >250um.

**Solubility:**

In water, the absorption of band-aid is bound alone by the bendability of the consistent band-aid which is action of the Kvalue.

**Dynamic viscosity**

The viscosities of water providone solution depend on Mol Wt. and concentrations of polymer used.

**Incompatibilities:**

Povidone form molecular adduct with sulfathiazole solutions, SA, Na- salicylates, tannin, Phenobarbitals and other compound.
Safety:

Povidone is broadly employed as an additive in articulate tablet and solution. It may be used as about controllable if taken orally back it is not transported from the GIT or close membrane. It does not have irritantion after effects on the skin and does not cause sensitization.

Applications in pharmaceutical formulations or technology:

- The PVP solutions are employed as binding agent in wet granulation process.
- The PVP is employed as a solubilizing agent in parenteral and oral formulation. It may also be used as coating agent.
- Widely known for its viscosity building capacity and used in pharmaceutical suspension, various solution and topical preparation

Used as:

- Carrier for drug (10-25%).
- Dispersing agent (5%).
- Eye drops (2-10%).
- Suspending agent (5%).
5.5: POLYETHYLENE GLYCOL 4000 (Indian Pharmacopeia 2007)

Synonyms: Macrogol 4000

Formula: \( \text{HOCH}_2 \left[ \text{CH}_2\text{OCH}_2 \right]_n \text{CH}_2\text{OH} \) where \( n \) is between 69 and 84.

Description: Polyethylene Glycol 4000 is a mixture of the polycondensation products of ethylene oxide and water obtained under controlled conditions. It is a Creamy white hard wax-like solid powder or flakes. The odor is faint and characteristic.

Category: Pharmaceutical aid (ointment base).

Solubility Description: Hydrophilic in nature and forming solution in CHCl₃.

Storage: Should store in tightly-closed containers.

STANDARDS

pH: Between 4.5 and 7.5, determined in aqueous medium.

Freezing point: Between 53° and 56°,

Hydroxyl value: Between 30 and 36, determined on 20 g.

Viscosity: Between 76 and 110 mm²s⁻¹, determined at 100° by Method A using a U-tube viscometer.
5.6. MATERIALS AND EQUIPMENTS

5.6.1 Materials

The drug, excipients, chemicals/reagents used for various experiments are enlisted as follows. AR label chemicals and reagent were used for study.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name Materials</th>
<th>Manufacturer/Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Atovaquone</td>
<td>Cadila pharmaceuticals India</td>
</tr>
<tr>
<td>2</td>
<td>Polyvinylprovidone K30 (PVP K30)</td>
<td>Sandoz Pvt. Ltd. Mumbai</td>
</tr>
<tr>
<td>3</td>
<td>PolyEthylene glycol 4000</td>
<td>Research-lab-Fine Chemicals Industries Mumbai</td>
</tr>
<tr>
<td>4</td>
<td>Methanol</td>
<td>Merck Ltd. Mumbai</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol</td>
<td>Loba Chemie, Mumbai</td>
</tr>
<tr>
<td>6</td>
<td>Concentration</td>
<td>Thomas Baker Chemicals</td>
</tr>
</tbody>
</table>
## Table 62: List of materials used

### 5.6.2: List of apparatus/ equipments/ instruments used

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Equipments/ Instruments</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Double beam Ultra Violet-visible absorption spectrometer</td>
<td>SMZ U-2900 Japan</td>
</tr>
<tr>
<td>2</td>
<td>Fourier Transform Infra-Red Spectrophotometer</td>
<td>Agilent carry 630 ATR</td>
</tr>
<tr>
<td>3</td>
<td>Hot-air Oven</td>
<td>Shital Scientific Industries</td>
</tr>
<tr>
<td>4</td>
<td>Disso-Test-App</td>
<td>Make- DTP- 06 P E.Lab</td>
</tr>
<tr>
<td>5</td>
<td>Electronic Weighing Balance (single pan)</td>
<td>Citizen, CY-104, Mumbai</td>
</tr>
</tbody>
</table>
5.7 EXPERIMENTAL WORK

5.7.1. Preformulation studies of selected drug

5.7.1.1. Organoleptic properties

Atovaquone is tested for organoleptic properties such as appearance, color, odor, taste, etc.

5.7.1.2. Melting point determination (Indian Pharmacopeia 2007)

Capillary technique was employed for determination of melting point of Atovaquone. Drug filled capillary was placed in the melting point apparatus containing silicon oil as a heating medium and the melting point was noted. Average of triplicate reading was taken.

5.7.1.3. Solubility determination (Indian Pharmacopeia 2007)

<table>
<thead>
<tr>
<th></th>
<th>Digital pH Meter</th>
<th>Hanna Instruments</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Differential Scanning Calorimeter</td>
<td>DSC200F3</td>
</tr>
<tr>
<td>8</td>
<td>X-Ray Differactometer</td>
<td>Philips PW 1710, Holland</td>
</tr>
</tbody>
</table>

Table 63: List of apparatus/ equipments/ instruments used
The “descriptive terms” is used to describe the approximate solubility of Pharmaceuticals (See Table below)

<table>
<thead>
<tr>
<th>Expressive terms</th>
<th>Part of solvent for each part of solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very-soluble</td>
<td>Less than 1</td>
</tr>
<tr>
<td>Freely-soluble</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Soluble</td>
<td>10 to 30</td>
</tr>
<tr>
<td>Sparingly-soluble</td>
<td>30 to 100</td>
</tr>
<tr>
<td>Slightly-soluble</td>
<td>100 to 1000</td>
</tr>
<tr>
<td>Very slightly-soluble</td>
<td>1000 to 10000</td>
</tr>
<tr>
<td>Practically insoluble or insoluble</td>
<td>Greater than or equal to 10,000</td>
</tr>
</tbody>
</table>

Table 64: Descriptive terms of solubility

Ethanol, dichloromethane, methanol and water was used to determine the solubility of pure Atovaquone molecule.

**Method**

Excess amount of drug was taken and dissolved in a measured amount of each solvent in a glass beaker to get a saturated solution. Solution was shaken intermittently to assist the attainment of equilibrium with the un-dissolved drug particles. Then measured quantity of the filtered drug solution was withdrawn after 24hrs and successively diluted with respective solvent and concentration was measured using U. V. Spectrophotometer at 252 nm for Atovaquone.

**5.7.1.4 Bulk Density** *(Alfred Martin et al., 2003; Aulton et al., 2002)*

Below mentioned formula was utilized for calculation of bulk densities.
5.7.1.5 Tapped Density (Alfred Martin et al., 2003, Aulton et al., 2002)

Below mentioned formula was utilized for calculation of tapped densities.

\[ D_o = \frac{M}{V_p} \]

Where,

\( D_o \) = Tapped bulk density
\( M \) = Weight of samples in grams
\( V_p \) = Final volumes of granules in cm³

5.8 Spectroscopic studies (Chandrakant et al., 2011)

5.8.1 UV Spectroscopy (determination of \( \lambda_{\text{max}} \))

5.8.1.1 UV spectra in acidic medium Atovaquone

The band-aid was kept in a alloyed silica cuvette 10 mm. UV spectrum was recorded in the ambit of 200-400 nm on Shimadzu 1700 UV-visible spectrophotometer at 1 cm aperture width.

5.8.2 IR spectrum interpretation (Chandrakant et al., 2011)
Atovaquone and proposed hydrophilic polymers and SD derivatives were studied for infrared spectroscopy. Samples were alloyed with potassium boiler (spectroscopy grade) and aeroembolism in to disk application hydraulic columnist afore scan from 4000-600 cm\(^{-1}\). The abstracts were observed on IR Solution software (version-1.10).

5.8.3 Standard calibration curve of Atovaquone

**Calibration curves of Atovaquone in 0.1N Hcl at \(\lambda_{\text{max}}\) 252nm**

The 0.1 normal hydrochloric acid is used as media to prepare the stock solution for checking the absorbance at \(\lambda_{\text{max}}\) 258 nm. The concentration in multiple of 2 µg/ml (Range 2,4….16 µg/ml)were made and their absorbances were measured with UV-Spectrophotometer (double-beam). Employing these absorbances, standard curves were obtained.

\[
Y = 0.0946 \\
R^2 = 0.9907
\]

Where

- \(Y\) is slope
- \(R^2\) is regression coefficient.

5.9 Preparation of solid dispersion of Atovaquone (Tantishaiyakul et al., 2006; Chandrakant et al., 2011)

5.9.1 Preparation of physical mixtures:

Physical Mixtures (PMs) of Atovaquone with water soluble carriers PEG-4000 and PVP-K 30 were manufactured by trituration essential quantity of drugs and carriers for five minutes in mortar, awaiting uniform mixture. The resultant mixture was screened
using # 80 sieve and kept in capped vial of glass at 25 °C. The drug and the polymers were blended with mortar and pestle.

5.9.2 Solid dispersion preparation:
Solid dispersion was manufactured by solvent evaporation technique. Atovaquone with polymers PEG-4000 and PVPK-30 taken in 1:1 ratio and then solubilized in adequate amount of ethanol with constant agitation. Then solvent was evaporated entirely with constant agitation to get dry powder by adjusting the temperature to 40-45°C. The ultimate solid powder was pulverized and crushed using mortar and pestle, then passed through 80 mesh sieve. Prepared solid dispersion stored in desiccators until used for further studies.

5.10 Characterization of solid dispersions of Atovaquone:
5.10.1 Micromeritics studies of solid dispersions.
Different Parameters like Density (Tapped & Bulk), flow property, compressibility (Carr’s Index), Hausner ration were used to characterize the SD.

5.10.1.1 Bulk density (Martin et al., 2005, Aulton et al, 2002)
The bulk densities were estimated by dividing mass of powders by bulk volume in cm³. 10 gm. of powder was carefully introduced into a 25ml graduated cylinder. The volume taken by the powder was calculated to determine bulk density.

Bulk densities were calculate by equation: [IP 1996]

\[ D_f = \frac{M}{V_p} \]
Where,

\[ D_f = \text{Loose bulk density} \]
\[ M = \text{Weight of samples in grams} \]
\[ V_p = \text{Final volumes of granules in cm}^3 \]

5.10.1.2 Tapped density (Martin et al., 2005, Aulton et al., 2002)

The tapped densities were calculated by division of the weight of powders by bulk volume in cm\(^3\). 10 gm. of powder was cautiously poured in a 25ml measuring cylinder. Initial volume of powder was noted and the measuring-cylinder was mechanically tapped 50 times, and reading of volume was taken. The tapped densities of all formulations were calculated by division of weight of powders in gram by the end tapped volume in cm\(^3\).

Tapped density was calculated by using equation: [IP 1996]

\[ D_o = \frac{M}{V_p} \]

Where,

\[ D_o = \text{Tapped bulk density} \]
\[ M = \text{Weight of samples in grams} \]
\[ V_p = \text{Final volumes of granules in cm}^3 \]

5.10.1.3. Carr’s index

Nowadays, powder’s flow characteristics can be predicted quickly and simply by studying compressibility index and Hausners ratio. The compressibility index is used as alternative measures of shape and size, bulk-density, surface area and moisture contents of material since all these properties could affect the obtained compressibility index.
The following equation was used to determine the compressibility of SD

\[
\text{Compressibility Index (CI)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100
\]

<table>
<thead>
<tr>
<th>Compressibility Index (%)</th>
<th>Flow property</th>
<th>Hausners-Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \leq 10 )</td>
<td>Excellent</td>
<td>1.000–1.110</td>
</tr>
<tr>
<td>11 – 15</td>
<td>Good</td>
<td>1.120–1.180</td>
</tr>
<tr>
<td>16 – 20</td>
<td>Fair</td>
<td>1.190–1.250</td>
</tr>
<tr>
<td>21 – 25</td>
<td>acceptable</td>
<td>1.260–1.340</td>
</tr>
<tr>
<td>26 – 31</td>
<td>Poor</td>
<td>1.350–1.450</td>
</tr>
<tr>
<td>32 – 37</td>
<td>Very poor</td>
<td>1.460–1.590</td>
</tr>
<tr>
<td>&gt; 38</td>
<td>Very-very poor</td>
<td>&gt;1.600</td>
</tr>
</tbody>
</table>

Table 65: Relationship between percentage compressibility and flowability

5.10.1.4. Hausner’s ratio

Following equation is used to calculate the Hausner ratio of a SD

\[ \text{Hausner Ratio} = \frac{\text{Tapped density}}{\text{Bulk Density}} \]

5.10.1.5 Angle of repose

It is used to differentiate the nature flowability of the solid (Table No. 4). It is a trait which relates to the inter-particulate frictions or struggle to movement among particle. It is the highest possible angle of surface of pile and the flat plane.
\[
\tan \theta = \frac{h}{r}
\]

\[
\theta = \tan^{-1} \frac{h}{r}
\]

Where,
- \( \theta \) = Angle of repose
- \( h \) = Height of pile
- \( r \) = Radius of pile

A funnel was set at a distance of approximately of 2-4cm over stand. The loose fine particles/granules were slowly passed down through funnel, until the cone of the powder produced. The angle of repose was determined by computing the heights of the pile and radius of the heap of powders. [Leon Lachman 1990]

<table>
<thead>
<tr>
<th>Flow-Properties</th>
<th>Angle of Repose (degree)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>25.0 –30.0</td>
</tr>
<tr>
<td>Good</td>
<td>31.0–35.0</td>
</tr>
<tr>
<td>Fair—aid not needed</td>
<td>36.0–40.0</td>
</tr>
<tr>
<td>Passable—can hangup</td>
<td>41.0–45.0</td>
</tr>
<tr>
<td>Poor—must vibrate, agitate</td>
<td>46.0–55.0</td>
</tr>
<tr>
<td>Very poor</td>
<td>56.0–65.0</td>
</tr>
<tr>
<td>Very-very poor</td>
<td>&gt;66.0</td>
</tr>
</tbody>
</table>

Table 66: Relationship between Angle of repose (\( \theta \)) and flowability

5.10.2 Percentage yield (i.e. recovery) of solid dispersion formed
(Tantishaiyakul et al., 2006)
The % recovery of formulated solid dispersion was resolute after complete removal of moisture. Thus % recovery calculation involves the weight of dried Solid dispersion to sum of the weight of drug and pharmaceuticals required for the formulation.

\[
\text{% Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and excipients}} \times 100
\]

5.10.3 Determination of drug content Atovaquone (Sushama et al., 2009; Aulton et al., 2002)

25 ml of acidic solution of Atovaquone was prepared using 0.1NHcl and 5mg of pure drug. One ml portion of stock solution was further dilute to 10ml with 0.1 N Hcl. The concentration of Ramipril was calculated by using the calibration curve.

5.10.4 Solubility study (Chandrakant et al., 2011)

Distilled water was used to measure the solubility of unadulterated Atovaquone, physical mixture and SD. The excess of samples were transferred to flask before adding distilled water. The mixtures then placed in mechanical shaker at 37° C for 48hr. The samples were assayed by double beam UV- spectrophotometer.

5.11 Solid state characterization of solid dispersions.

5.11.1 Drug and excipients interaction study (FTIR) (Chandrakant et al., 2011).

Infrared spectrum of pure drug, PM and hydrophilic polymers were determined using Shimadzu Model-8400S. The wavelength selection range for analysis was set in the range of 4000-400 cm\(^{-1}\).

5.11.2 Differential-scanning-calorimetry: (Chandrakant et al., 2011)

The DSC studies of samples were done to check the thermal behavior of the
formulation. The process is carried out on DSC-60 instrument, Shimadu, Japan. The standard operative procedure involves the loading of sample into aluminum pans and crimper was used to crimp the lid. The parameters used for this investigation are 1) medium – nitrogen 2) Heating Rate -10°C per minute 3) Covering-temperature range 25 to 300°C. The DSC equipment was calibrated as per NPL guideline using internal standard. Assay of abstraction was conducted on TA-60WS (thermal analysis software).

5.11.3 X-Ray diffraction analysis (XRD) (Chandrakant et al., 2011)

The x-ray diffractometer (Philips PW 1710, Holland) were used to study the diffraction pattern of the different formulations. The scan rate was fixed at 1°/min. and 2θ scan range was maintained.

5.11.4 Micromeritics studies of mixture blend

5.11.4.1. Bulk density and Tapped density:

Is determined by placing the 5 gm preparation in 50 ml calibrated cylinder and the fluff volume measured. Then cylinder was tapped 100 time by maintain uniform altitude. Then resultant volume was recorded

By using the equation, both bulk density & tapped density was determined.

\[ \text{Density} = \frac{\text{Mass}}{\text{volume}} \]

\[ \text{Bulk volume} = \frac{5\text{gm}}{\text{bulk volume}} \]

\[ \text{Tapped volume} = \frac{5\text{gm}}{\text{tapped volume}} \]

5.11.4.2. Carr’s index (percentage compressibility)

Following equation is used to calculate the flowability of granules.

\[ \text{Compressibility Index (CI)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \]

5.11.4.3. Hausner’s ratio

Following equation is used to calculate
Hausner Ratio  = \frac{\text{Tapped density}}{\text{Bulk Density}}

5.11.4.4. Angle of repose (Flowability)

Flowability of pharmaceuticals is calculated in term of angle of repose. Which is outlined as angle getable between horizontal plane and therefore the surface of pile. Usually the funnel methodology is employed to work out the flowability. This methodology involve the passing the developed granules through the passageway of the funnel on surface. That led to formation of pile on the surface. Live the peak of pile \(h\) and radius of pile \(r\). Substituting these value within the below equation provides the angle of repose.

\[
\tan \theta = \frac{h}{r}
\]

\[
\theta = \tan^{-1} \left( \frac{h}{r} \right)
\]

Where,

\(\theta\) = Angle of repose
\(h\) = Height of pile
\(r\) = Radius of pile

5.11.4.5 In-vitro dissolution test for Atovaquone solid dispersion:

Firstly the pure drug, developed physical mixture and solid dispersions were filled in to the capsule and were tested for In-Vitro dissolution study by utilizing USP type-2 Dissolutions Testing Apparatus (6 vessel assembly, Paddle-type II) at fifty revolutions per minute. 900ml of 0.1N HCL solution was used as dissolving media. Temperature was kept 37±0.5°C. Samples of 5ml were taken at 5 minute time intervals & same amount of pure dissolution fluid preserved at identical temperatures was reinstated to maintain sink conditions. Samples were passed from a whatman filter-paper, suitably
diluted using 0.1N HCL solutions and examined spectrophotometricaly at 252nm. [USP, 2004]

Dissolution conditions used in the study are indicated below:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of rotation</td>
<td>100 rpm</td>
</tr>
<tr>
<td>Temperature</td>
<td>37°C ± 0.5°C</td>
</tr>
<tr>
<td>Sampling interval</td>
<td>5 min</td>
</tr>
<tr>
<td>Test Medium</td>
<td>0.1N HCL</td>
</tr>
<tr>
<td>Volume of test medium</td>
<td>900 ml in each vessel.</td>
</tr>
<tr>
<td>Vol of sample taken</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

Table 67: Parameter of in-vitro dissolution test for Atovaquone and its solid dispersion

5.12 SEM (Scanning Electron Microscopy)

The detailed surface characteristics of the Atovaquone drug and their solid dispersion were observed by a scanning electron microscope (Model: JEM-100S, Jeol,Tokyo,Japan).
5.13 RESULT AND DISCUSSION

5.13.1 RESULTS

5.13.1.1 Preformulation study.

Physical characters of Atovaquone were found as under.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Characters</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nature</td>
<td>Crystalline powder</td>
</tr>
<tr>
<td>2</td>
<td>Color</td>
<td>Dark Yellow</td>
</tr>
<tr>
<td>3</td>
<td>Odor</td>
<td>Odorless</td>
</tr>
<tr>
<td>4</td>
<td>Taste</td>
<td>Slightly Bitter</td>
</tr>
<tr>
<td>5</td>
<td>Melting point</td>
<td>219-221°C</td>
</tr>
<tr>
<td>6</td>
<td>Solubility-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In methanol</td>
<td>Soluble</td>
</tr>
<tr>
<td></td>
<td>In water</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td></td>
<td>In ethanol</td>
<td>Freely soluble</td>
</tr>
<tr>
<td>7</td>
<td>Density (Bulk)</td>
<td>0.217 gm /cm³</td>
</tr>
<tr>
<td>8</td>
<td>Density (Tapped)</td>
<td>0.385 gm /cm³</td>
</tr>
</tbody>
</table>

5.13.1.2 Solid dispersions Preparation:

The SD formulations of Satranidazole drug were prepared using SET methodology (solvent evaporation technique).

5.13.1.3 Characterization of solid dispersion of Atovaquone:
Atovaquone solid dispersions were characterized by following parameters.

1. Micrometrics studies
2. Percentage yield of solid dispersion
3. Drug contents
4. Solubility analysis
5. *In vitro* dissolution studies
6. XRD
7. SEM
8. DSC

### 5.13.1.4 Micromeritics studies

The results of Micromeritics properties of solid dispersion formulations were as below

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Formulation code</th>
<th>Bulk densities (g/cm³)*</th>
<th>Tapped density (g/cm³)*</th>
<th>Percentage Compressibility index</th>
<th>Hausners ratio</th>
<th>Angle of repose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>0.217</td>
<td>0.385</td>
<td>43.48</td>
<td>1.77</td>
<td>46°25'</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>0.323</td>
<td>0.327</td>
<td>0.968</td>
<td>1.10</td>
<td>27°15'</td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>0.333</td>
<td>0.370</td>
<td>1.00</td>
<td>1.11</td>
<td>30°14'</td>
</tr>
</tbody>
</table>

### 5.13.1.5 Percentage yield

The results of percentage yield of binary solid dispersion formulations were as below

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation code</th>
<th>Percentage yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B1</td>
<td>79.91</td>
</tr>
<tr>
<td>2</td>
<td>C1</td>
<td>75.44</td>
</tr>
</tbody>
</table>

Table 69: Percentage yield of solid dispersion formulations
5.13.1.6 Drug contents

Table showing the % drug content observed in pure molecule and SD formulations.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Formulations code</th>
<th>Percent drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B1</td>
<td>96.83</td>
</tr>
<tr>
<td>2</td>
<td>C1</td>
<td>89.46</td>
</tr>
</tbody>
</table>

Table 70: % drug content of SD formulations

5.13.1.7 Solubility studies:

Table showing the outcome of Solubility study of binary SD formulations

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Formulation code</th>
<th>Solubility(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1</td>
<td>12.657</td>
</tr>
<tr>
<td>2</td>
<td>B1</td>
<td>40.880</td>
</tr>
<tr>
<td>3</td>
<td>C1</td>
<td>22.789</td>
</tr>
</tbody>
</table>

Table71: Solubility (µg/ml) of pure drug and solid dispersion formulation

5.14  *In vitro* dissolution studies of solid dispersion formulations

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Percentage of drug release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0.740</td>
</tr>
<tr>
<td>10</td>
<td>4.08</td>
</tr>
<tr>
<td>15</td>
<td>8.97</td>
</tr>
</tbody>
</table>
Table 72: % Drug release of pure drugs and solid dispersion formulations

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A1</th>
<th>B1</th>
<th>C1</th>
<th>D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>18.72</td>
<td>29.79</td>
<td>35.41</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>20.01</td>
<td>33.83</td>
<td>49.22</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>24.96</td>
<td>43.16</td>
<td>45.44</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>26.43</td>
<td>54.48</td>
<td>49.49</td>
<td></td>
</tr>
</tbody>
</table>

Figure 54: *In-vitro* dissolution profile of Atovaquone and its solid dispersion formulations

5.15 Spectroscopic studies

5.15.1 UV- spectroscopy (determination of \( \lambda_{\text{max}} \)) of Atovaquone:

- \( \lambda_{\text{max}} \) of Atovaquone in pH 1.2 (0.1 N HCl) found to be 252 nm.
- UV-spectrum of Atovaquone was shown in figure 55.
- Calibration curve was drawn, which follows beer’s Lambert law (Figure 56).

UV-spectrum of Atovaquone in 0.1 N Hydrochloric acid (pH 1.2)
Figure 55: UV-spectrum of Atovaquone in 0.1N Hydrochloric acid

Figure 56: Calibration curve of Atovaquone pure drug

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table No.73: Calibration curve of Atovaquone pure drug

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.136</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.392</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.523</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.752</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.982</td>
</tr>
</tbody>
</table>

**IR spectroscopy**
Results of FTIR spectroscopy were as below
  a) **IR spectra of A1 sample:**
Figure 57: IR spectra of A1 sample
b) IR spectra of B1 sample:

Figure 58: IR spectra of B1 sample
c) IR spectra of C1 sample:
d) IR spectra of Physical-mixture of Atovaquone with PEG4000:
Figure 60: IR spectra of physical mixture Atovaquone with PEG4000.
e) IR spectra of Physical-mixture of Atovaquone with PVPK30:

Figure 61: IR spectra of Physical-mixture of Atovaquone with PVPK30
5.16 Differential scanning calorimetry:
   a) DSC of sample A1:
Figure 62: DSC of sample A1

b) DSC of Sample B1:
Figure 63: DSC of sample B1
c) DSC of sample C1:

Figure 64: DSC of sample C1
5.17 X-ray diffraction analysis:

a) XRD of Sample A1:
Figure 65: XRD of sample A1

b) XRD of sample B1:
Figure 66: XRD of sample B1
c) XRD of sample C1:

Figure 67: XRD of sample C1
5.18. Scanning Electron Microscopy:

a) SEM of sample A1:

Figure 68: SEM of sample A

b) SEM of sample B1:
f) SEM of sample C1:
5.19 DISCUSSION

5.19.1 Preformulation study

The physical characteristic of Atovaquone was found to be identical with standards given in analytical profile of drug substances.

5.19.2 Spectroscopic studies

5.19.2.1 UV- spectrum of Atovaquone

The $\lambda_{\text{max}}$ of Atovaquone was found to be at 252 nm (at 1.2 pH). This complies with the standards. On the basis of the above tests, it was confirmed that the drug sample of Atovaquone was an authentic one.

5.19.2.2 Calibration curve of Atovaquone in 0.1N HCl:

Equation of line ($y = mx + c$) of calibration curve of Atovaquone in 0.1N HCl (pH1.2) was,

$$Y = 0.0946X + 0.0003$$

$R^2 = 0.9907$

Where,

$M =$ slope of the line

$C =$ constant
5.19.3 Characterization of solid dispersion of Atovaquone:

5.19.3.1 Micromeritic studies

5.19.3.2 Percentage yield:

The % yield was found good with formulated SD. The Batch B-1 showing maximum yield of 79.9%. For the rest of batches the overall % yield is more than 71.85%.

5.19.3.3 Drug content:

All SD formulation were tested for % drug content and found in the rage 96.55-98.45% and mentioned in table No.70

5.19.3.4 FTIR-spectroscopy

Figure 57-61 showing the IR spectral data of authentic Atovaquone and the polymer used for formulation of SD mixture. The FTIR spectra of authentic Atovaquone was matching for the chapped crumb and for crumb acquired afterwards dehydration in the biologic band-aid acclimated to adapt the solid dispersion.

5.19.3.4.1 IR spectrum of sample A1:

These spectra showed the characteristic peaks of Atovaquone which occurred at 3366, 2925, 2854, 1589 and 1654 cm\(^{-1}\). This is similar to the previously recorded spectra of the pure drug which corresponds to O-H, Aromatic ring, double bond=o resp.

5.19.3.4.2 IR spectrum of sample B1:

These spectra showed the characteristic peaks of Atovaquone which occurred at 3374, 3883, 2858 and 1594 cm\(^{-1}\). This is similar to the previously recorded spectra of the pure drug.
5.19.3.4.3 IR spectrum of sample C1:
This FTIR spectrum showed the characteristic peak of Atovaquone at 3369, 2923, 2853, 1594 cm\(^{-1}\) which is identical to the pure drug.

5.19.3.4.4 IR spectra of physical-mixture of Atovaquone with PEG4000:
The FTIR spectra of the physical-mixture of the Atovaquone with PEG 4000 had shown the characteristic peak at the 3371, 2924, 2854, 1590 and 1624 cm\(^{-1}\).

5.19.3.4.5 IR spectra of physical-mixture of Atovaquone with PVPK30:
The Spectra of PM (physical-mixture) of Atovaquone with PVPK30 had shown the characteristic peak at 3370, 2925, 2853, 1624 and 1654 cm\(^{-1}\).

5.20 Solubility study
The result of solubility study indicates that pure drug has a very low solubility. The solubility of drug in the solid dispersion enhanced considerably indicating that the inclusion of PEG-4000 increases the solubilities than PVP K30. This may be due to capacity of PEG-4000 to avoid recrystallization during cooling of solid dispersion. As PEG-4000 concentration increases the solubility also increases, the maximum solubility was observed in B1 formulation.

5.20.1 In vitro dissolution of solid-dispersions:
The solid dispersion of PEG4000 showed increase in drug dissolution than PVP k30. There is increase in the rate of dissolution and solubilities by using PEG-4000 in solid dispersions of Atovaquone with 2-4 folds.

5.21. Differential-scanning-calorimetry
5.21.1 DSC of SampleA1:
The pure Atovaquone showed a characteristic endothermic melting peak at $221^\circ$ indicating that the drug Atovaquone highly crystalline in nature.

5.21.2 DSC of Sample B1:

The DSC curve of solid dispersion prepared by solvent evaporation method exhibit broad endotherm peak was significantly reduced to $61^\circ$ c. The result confirms that the Atovaquone with PEG 4000 exist in the crystalline nature and in the optimized SD formulation.

5.21.3 DSC of Sample C1:

The DSC curve of solid dispersion of Atovaquone with PVP K30 shows reduced in the intensity of the endothermic peak to $192^\circ$ c. It shows compatibility with the drug.

5.22 X-ray diffraction analysis

5.22.1 XRD of Sample A1:

The characteristic peaks of Atovaquone appeared in the 2$\theta$ range of 20-25$^\circ$, which indicates that, the unprocessed Atovaquone was a crystalline material.

5.22.2 XRD of Sample B1:

The characteristic peaks of solid dispersion of Atovaquone with PEG4000 appeared in the 2$\theta$ range of 20-25$^\circ$.

5.22.3 XRD of Sample C1:

The characteristic peaks of solid dispersion of Atovaquone with the PVP K30 appeared in the 2$\theta$ range of 20-25$^\circ$, which indicates that, the pure Atovaquone was a crystalline substance.
5.23 SEM (Scanning Electron Microscopy):

The figure 68-70 showing the result of SEM of Atovaquone and SD formulation with PVPK30, PEG4000. The study clearly reveals the difference between SEM of Atovaquone and its SD formulation. Also observed definite morphological changes in crystal of drug.

5.24 SUMMARY AND CONCLUSION

Drug dissolution is the rate limiting step for peroral-bioavailability of poor aqueous soluble drug that consequently affects the *in vivo* drug absorption. Solubility is the key factor for oral absorption of poor aqueous soluble drug. Atovaquone is poorly soluble drug and shows poor bio-availability. Thus lots of approaches tried to enhance its water solubilities and its rate of dissolution from different drug delivery system and in addition improvement in flow properties for ease in compression more are under constant investigation. In this work, solid dispersions method was assessed for solubility and rate of dissolution improvement.

Therapeutic efficiency of drugs is dependent on the bio-availability and eventually on drug molecules solubilities. The products obtained by all these means were appropriately characterized and evaluated for enhancement of solubility and for their *in-vitro* dissolution

Atovaquone is a potent antiprotozoal agent having high lipophilicity and poor aqueous solubility. Shows slow and variable absorption when administered orally. Thus the purpose of the work was to design solid dispersion of the drug, to achieve high rate dissolution and enhancement of its oral bioavailability.

Solid dispersion prepared with hydrophilic polymer showed a higher enhancement in solubility rate with PEG4000 i.e. 2-3 folds as compared to 1.2 fold for that prepared with PVPk30. The DSC data indicated a depression in melting temperature and enthalpy for the formulation. The XRD results indicated no modification in crystal structure of drug in formulation. The Lack of chemical interactions among carrier and drug was confirmed by the FTIR spectra. The *in-vitro* dissolution studies revealed a considerable boost in dissolution rate of SDs of Atovaquone in contrast to pure drug.
Thus from studies, it could be concluded that SDs of poor aqueous soluble Atovaquone by solvent evaporation technique were effectively formulated using PEG-4000 and PVP-K 30 hydrophilic polymers. Thus, the statement can be given that the rate of dissolution and solubility of poor aqueous soluble Atovaquone can be appreciably improved by solid dispersion by use of water soluble carriers by solvent evaporation technique.