6.3.1 PREPARATION OF POLYHERBAL FORMULATION (PHF)

The polyherbal formulation (capsules) contained the ethanolic extracts of leaves of Gymnema sylvestre, fruits of Momordica charantia, rhizomes of Curcuma longa, seeds of Eugenia jambolana and fruits of Embilica officinalis were prepared in the different ratio (as mentioned in table 3). The quality of the polyherbal formulation was tested as per the WHO guidelines for the quality control of herbal materials. As per the guidelines, specific tests such as sampling, ash content, extractable matter, foaming index, loss on drying, foreign matters and specific powder characteristic tests such as angle of repose and bulk density were undertaken and significant results were recorded. (Mishra Uma Shankar et al, 2011)

6.3.2. Preparation of formulation (PHF: Capsule) by wet granulation method (Mishra Uma Shankar et al, 2011)

The formulation preparation began with trials by adding a different ratio of binders and selecting the quantity of lubricants and preservatives, and finally the procedure was optimized. Ethanolic extracts of leaves of Gymnema sylvestre, fruits of Momordica charantia, rhizomes of Curcuma longa, seeds of Eugenia jambolana and fruits of Embilica officinalis were finely powdered (sieve 40), and mixed in the ratio as mentioned in Table-8 and taken for the preparation of capsules by wet granulation technique using lactose solution as a binder. The wet mass was passed through sieve number 22 to obtain granules. The granules were dried at 45°C in a tray dryer. The granules were lubricated with magnesium stearate. Diluents and preservatives were added. After this, the granules from the optimized batch were filled in capsules colored yellow-red of size “0” in a capsule filling machine. The capsules were then deducted and transferred into poly bags, labeled, and the samples were evaluated as per the testing requirements. Each 500 mg of herbal
capsule contained the extracts of *Gymnema sylvestre*, *Momordica charantia*, *Curcuma longa*, *Eugenia jambolana*, *Embilica officinalis* and lactose and excipients– quantity sufficient (q.s.) (Table-8).

**Table 3: Preparation of Poly Herbal Formulation (Capsule)**

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Ingredients</th>
<th>Quantity (in mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1   F2  F3  F4  F5  F6  F7</td>
</tr>
<tr>
<td>1.</td>
<td>EEGSL</td>
<td>25   25  50  25  75  75  100</td>
</tr>
<tr>
<td>2.</td>
<td>EEMCF</td>
<td>25   50  100 50  75  100 75</td>
</tr>
<tr>
<td>3.</td>
<td>EECLR</td>
<td>25   25  50  100 75  75  100</td>
</tr>
<tr>
<td>4.</td>
<td>EEEJS</td>
<td>25   50  100 50  75  100 75</td>
</tr>
<tr>
<td>5.</td>
<td>EEEOF</td>
<td>25   25  50  100 75  75  100</td>
</tr>
<tr>
<td>6.</td>
<td>Lactose</td>
<td>200  100 50  100 75  25  25</td>
</tr>
<tr>
<td>7.</td>
<td>Magnesium sterate</td>
<td>100  200 50  50 25  25  12.5</td>
</tr>
<tr>
<td></td>
<td>Net Weight</td>
<td>500  500 500 500 500 500 500</td>
</tr>
</tbody>
</table>
6.3.3. **Preformulation studies** (Lachman- Liberman 1999, USP 2007)

Preformulation parameters such as bulk density, tap density, Carr's index, Hausner's ratio, and angle of repose were determined for the laboratory granules.

### 6.3.3.1. Bulk density

Bulk densities were determined by pouring gently 25 gm of sample through a glass funnel into a 100 ml graduated cylinder. The volumes occupied by the sample were recorded. Bulk density was calculated by the using following formula:

\[
\text{Bulk density (g/ml) = weight of sample in gms/ volume occupied by the sample}
\]

### 6.3.3.2. Tapped density

Tapped densities were determined by pouring gently 25 gm of sample through a glass funnel into a 100 ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping were recorded and tapped density was calculated.

\[
\text{Tapped density (g/ml) = weight of sample in gms/ volume occupied by the sample}
\]

### 6.3.3.3 Compressibility index

It is also one of the simple methods to evaluate flow property of powder by comparing the bulk density and tapped density. A useful empirical guide is given by the Carr’s compressibility.

\[
\text{Carr's index= TD-BD/TDX100}
\]
Table 4: Grading of powders for their flow properties

<table>
<thead>
<tr>
<th>Consolidation index (Carr’s index)</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>Excellent</td>
</tr>
<tr>
<td>15-16</td>
<td>Good</td>
</tr>
<tr>
<td>*18-21</td>
<td>Fair to Passable</td>
</tr>
<tr>
<td>*23-35</td>
<td>Poor</td>
</tr>
<tr>
<td>33-38</td>
<td>Very poor</td>
</tr>
<tr>
<td>&lt;40</td>
<td>Very Very poor</td>
</tr>
</tbody>
</table>

6.3.3.4. Hausner ratio

It provides an indication of the degree of densification which could result from vibration of the feed hopper.

**Hausner ratio = Tapped density/ Bulk density**

Lower Hausner ratio = Better flow ability, Higher Hausner ratio = Poor flow ability

6.3.3.5. Angle of repose

Flow properties of the physical mixtures of all the formulations were determined by calculating angle of repose by fixed height method. A funnel with 10 mm inner diameter of stem was fixed at a height of 2 cm. over the platform. About 10 gm of sample was slowly passed along the wall of the funnel till the tip of the pile formed
and touches the steam of the funnel. A rough circle was drawn around the pile base and the radius of the powder cone was measured. Angle of repose was calculated from the average radius using the following formula.

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

Where, \( \theta \) = Angle of repose, \( h \) = Height of the pile, \( r \) = Average radius of the powder cone

**Table 5: Relationship between angle of repose (\( \theta \)) and powder flow**

<table>
<thead>
<tr>
<th>Angle of repose (( \theta ))</th>
<th>Type of flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>Excellent</td>
</tr>
<tr>
<td>25-30</td>
<td>Good</td>
</tr>
<tr>
<td>*30-40</td>
<td>Passable</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Very poor</td>
</tr>
</tbody>
</table>

*Adding the glidant e.g. 0.2% aerosol, may improve flow


The polyherbal capsules were evaluated for their description, microbial load, uniformity of dosage units, weight variation, disintegration time, and moisture content, and compared with Indian pharmacopoeial standards.
6.3.4.1. Organoleptic Characters

The PHF (Capsule) were examined for their color and appearance. The color, odor, taste were observed and noted down.

6.3.4.2. Moisture content

Moisture content was determined by using automatic Karl Fischer titration apparatus.

6.3.4.3. pH

The pH were determined by pH meter.

6.3.4.4. Weight variation

Twenty capsules were individually weighed and the average weight of the capsule was calculated. The individual weights of the each capsule should be within the limits of 90% and 110% of the average weight.

6.3.4.5. Disintegration time

Disintegration test was performed using the digital microprocessor based disintegration test apparatus (Electro lab, Mumbai, India). One capsule was introduced into each tube and a disk was added to each tube. The assembly was suspended in water in a 1000 ml beaker. The volume of water at its highest point was at least 25 mm below the surface of the water and at its lowest point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained at a temperature of 37 ± 2°C.

6.3.4.6. Drug content

Five randomly selected capsules were weighed, removed the cap and body and were powdered. The powdered equivalent to 100 mg drug in one capsule was taken and
transferred in a 100 ml flask containing 100 ml of 0.1 N HCl pH 1.2. The flask was shaken on a flask shaker and was kept for few hours for the sedimentation of undissolved materials. The solution is filtered through Whatman filter paper. 10ml of this filtrate was taken and appropriate dilution was made. The samples were analyzed at specific wavelength using UV visible spectrophotometer. The drug content was determined from the standard curve prepared at optimum $\lambda$ max.

6.3.4.7. Drug Release

Drug release was assessed by dissolution test under the following conditions: $n = 6$ (in triplicate), USP type II dissolution apparatus (Lab India, DISSO 2000) at 50 rpm in 900 ml of 0.1N HCl pH1.2 maintained at 37 ± 0.5°C. The tablet was allowed to sink to the bottom of the flask before stirring. Special precaution was taken not to form air pockets on the surface of the tablet. Five milliliters of the sample was withdrawn by using a syringe filter at regular intervals and replaced with the same volume of pre warmed (37 ± 0.5°C) fresh dissolution medium. The drug content in each sample was analyzed after suitable dilution using UV spectrophotometer method at respective maximum wavelength.

6.3.4.8. Stability Studies

The success of an effective formulation can be evaluated only through stability studies. The purpose of stability is to obtain a stable product which assures its safety and efficacy up to the end of shelf life at defined storage conditions and peak profile.

The optimized formulation of the drug was subjected to accelerated stability studies at specified conditions of temperature and relative humidity of 25°C/60% RH, 30°C/60% RH and 40°C/75% RH for 3 months. After the completion of three month the samples were analyzed visually for any color changes due to physical
and chemical interaction within excipients and with the drug. The percentage drug content in all the tablets was determined after specified period (ICH guidelines 1993).

### 6.3.4.9. Microbial load analysis

For the safe use of the polyherbal capsules, microbial count was done and it was checked whether the total aerobic viable count, yeasts and molds were within the prescribed limits and the microorganisms, *Escherichia coli*, *Clostridia*, *Salmonellae*, *Shigella*, *Pseudomonas*, and *Staphylococcus*, were absent in the final (optimized) formulation (Tyler VE 1994).

### 6.3.5. Formulation of Vati (Marwick C 1995)

Vati were prepared by method namely sompakvidisurya - pakvidi, wet granulation, dry granulation direct compression. Formulation of tablet is indicated in [Table 3.4]. Initially jamun powder admixture was prepared by mixing amala, haldi, karela powder and gudmar in the ratio mentioned in table 6. The additives such as talc, mg state and preservatives were added to gain the weight of vati to 100 mg.
Table 6: Formula for Ayurvedic Vati

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Ingredients</th>
<th>Part used</th>
<th>Quantity (in mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>1.</td>
<td>Gymnea sylvestre</td>
<td>Leaves</td>
<td>50</td>
</tr>
<tr>
<td>2.</td>
<td>Momordica charantia</td>
<td>Fruits</td>
<td>10</td>
</tr>
<tr>
<td>3.</td>
<td>Curcuma longa</td>
<td>Rhizomes</td>
<td>10</td>
</tr>
<tr>
<td>4.</td>
<td>Eugenia jambolana</td>
<td>Seeds</td>
<td>10</td>
</tr>
<tr>
<td>5.</td>
<td>Embilica officinalis</td>
<td>Fruits</td>
<td>10</td>
</tr>
<tr>
<td>6.</td>
<td>Talc</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>7.</td>
<td>Mg sterate</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>8.</td>
<td>Methyl Paraben</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Net Weight</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

6.3.6. Evaluation of Formulated vati (British Herbal Medicine, 1996, Bhutani KK 2006.)

Methods

- Morphological evaluation
- Pharmaceutical evaluation

6.3.6.1. Morphological Evaluation:

The prepared vati were observed for the organoleptic characters e.g colour, odour, size, shape, and taste.
6.3.6.2. Pharmaceutical Analysis:

6.3.6.2.1. Friability Test

The Roche friability test apparatus was used. 20 tablets were weighed collectively and transferred to the friabilator. The friabilator was rotated for 4 minutes at 25 rpm, tablets were taken out and dust was removed. Tablets were again weighed and %loss was calculated.

\[ \% \text{ friability} = \frac{a-b}{a} \times 100 \]

a= collection weight before friability and

b= collective weight after friability.

6.3.6.2.2. Weight Variation Test

20 tablets were taken and weighed individually. Calculated average weight and compared the individual tablet weight to the average. The tablet pass the U.S.P test if not more that 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit.

Maximum percentage difference for tablets weighing 130-324mg is ± 7.5% and for tablets weighing more than324mg is ±5%.

6.3.6.2.3. Hardness

Tablet requires a certain amount of strength or hardness and resistance to friability to withstand mechanical shakes of handling in manufacture, packaging and shipping. Hardness generally measures the tablet crushing strength. Hardness of 10 tablets was checked by the monsento hardness tester and average hardness was calculated.
6.3.6.2.4. Disintegration Test

The USP device to test disintegration uses 6 glass tubes that are 3” long; open at open at the top and 10 mesh screen at the bottom end. To test for disintegration time, one tablet is placed in each tube and the basket rack is positioned in a 1-L beaker of water, simulated gastric fluid or simulated intestinal fluid at 37±20 °C such that the tablet remain 2.5 cm below the surface of liquid on their upward movement and not closer than 2.5 cm from the bottom of the beaker in their downward movement, move the basket containing the tablets up and down through a distance of 5-6cm at a frequency of 28 to 32 cycles per minute. Floating of tablets can be prevented by placing perforated plastic discs on each tablet. According to the test the tablet must disintegrate and all particles must pass through the 10 mesh screen in the time specified. If any residue remains, it must have a soft mass.

6.3.6.2.5. Dissolution Test

Phosphate buffer solution of pH 7.5 was made. Then, 6.8gm of potassium dihydrogen ortho phosphate and 1.56 gm of sodium hydroxide were dissolved in 900 ml of water & adjust the pH to 7.5 with sodium hydroxide solution and dilute with water to produce 1000ml. Then 100ml buffer solutions were taken separately and pour the remaining 900ml in the dissolution apparatus. After this, the sample was taken out at different time intervals as 5min, 10min, 20min, 40 min. After taking the sample the absorbance was checked.

6.3.6.2.6. Stability Studies

The success of an effective formulation can be evaluated only through stability studies. The purpose of stability is to obtain a stable product which assures its safety and efficacy up to the end of shelf life at defined storage conditions and peak profile.
The optimized formulation of the drug was subjected to accelerated stability studies at specified conditions of temperature and relative humidity of 25°C/60% RH, 30°C/60% RH and 40°C/75% RH for 3 months. After the completion of three month the samples were analyzed visually for any color changes due to physical and chemical interaction within excipients and with the drug. The percentage drug content in all the tablets was determined after specified period (ICH guidelines 1993).

6.3.6.2.7. Modeling of Dissolution Profiles of Optimized formulation

In vitro dissolution has been recognized as an important element in drug development under certain assessment of Bioequivalence. Several theories/kinetics models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where \( f_t \) is a function of ‘t’ (time) related to the amount of drug dissolved form the pharmaceutical dosage system (Costa and Lobo 2001). Whenever a new solid dosage form is developed or produced, the drug release/dissolution from solid pharmaceutical dosage form is necessary to ensure that the drug dissolution occurs in an appropriate manner. Several theories/kinetic models describe drug dissolution form immediate and modified release dosage. These represents the drug dissolution profiles where \( f_t \) is a function of ‘t’ (time) related to the amount of drug dissolved from the pharmaceutical dosage forms.

The quantitative interpretation of the value obtained from the dissolution assay is facilitated by mathematical equation which translates the dissolution curve in function of some parameters related with the pharmaceutical dosage forms.

In the present study, data of the \textit{in vitro} release were fitted to different equations and kinetic models to explain the release kinetics from the matrix tablets. The kinetic
models used were a Zero order equation, First order, Hickson-Crowell, Higuchi release and Korsmeyer-Peppas models.

The dissolution release kinetics and result of best fit model among the preparations were also compared. To study the mechanism of drug release from the optimized formulation of matrix tablets, the release data were fitted to the following equations:

**Zero-order equation:** 
\[ Q_t = Q_0 + k_0 t \]

Where,
- \( Q_t \) is the amount of drug release in time \( t \),
- \( Q_0 \) is the initial amount of drug in the solution (most times, \( Q_0 = 0 \)),
- \( k_0 \) is the zero order release rate.

**First-order equation:** 
\[ \ln Q_t = \ln Q_0 + k_1 t \]

Where,
- \( Q_t \) is the amount of drug released in time \( t \),
- \( Q_0 \) is the initial amount of drug in the solution,
- \( k_1 \) is the first order release rate constant.

**Higuchi’s equation:** 
\[ Q = k_H t^{1/2} \]

Where,
- \( Q \) is the amount of drug release at time \( t \),
- \( k_H \) is the Higuchi diffusion rate constant.

**Korsmeyer equation:** 
\[ \frac{M_t}{M_{\infty}} = K t^n \]

Where,
- \( M_t \) is the amount of drug released at time \( t \),
- \( M_{\infty} \) is the amount of drug released after infinite time,
- k is a kinetic constant incorporating structural and geometric characteristics of the tablet,
- n is the diffusion exponent indicative of the drug release mechanism.

The mechanism of drug release was dependent on the value of ‘n’.

**Table 7: Value of ‘n’ and corresponding mechanism of drug release**

<table>
<thead>
<tr>
<th>Value of ‘n’</th>
<th>Mechanism of drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 0.5</td>
<td>Case – I (Fickion) diffusion or square root of time kinetics</td>
</tr>
<tr>
<td>0.5 &lt; n &lt; 1</td>
<td>Anomalous (non-Fickion) diffusion</td>
</tr>
<tr>
<td>n = 1</td>
<td>Case – II transport</td>
</tr>
<tr>
<td>n &gt; 1</td>
<td>Super Case – II transport</td>
</tr>
</tbody>
</table>