FORMULATION AND EVALUATION OF CARICA PAPAYA NANOEMULSION FOR TREATMENT OF DENGUE AND THROMBOCYTOPENIA

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ABSTRACT

Nanoemulsions are oil-in-water dispersions, having consists of oil, water, and S_{mix} (surfactant and co-surfactant mixture) of dispersion with size range of 20 to 200 nm. These are biphasic system in which one phase is disperse in the other phase, in the form in minute droplets Chime S.A. et al. [2014]. Nanoemulsion is biphasic dispersion, consists of oil phase, aqueous and higher proportion of surfactant and co-surfactant mixture of which yield size of nanoemulsion in the range of 50 to 200 nm. These systems possess intimately dispersed phase into another phase. These are two types of nanoemulsion such as oil/water (o/w) or water/oil (w/o) and bicontinuous Mason T.G. et al. [2006]. The dispersed phase is also known as internal phase or discontinuous phase, while the outer phase is called external phase or continuous phase. These are two types of nanoemulsion such as oil in water type (o/w) or water in oil type (w/o) and bicontinuous. It they appear transparent due to low size range Mishra R.K. et al. [2014]. It can also be defined as that nanoemulsions are thermodynamically stable, isotropically clear dispersion and stabilized by an interfacial film called as surfactant molecule. Also, it is known as miniemulsions, submicron emulsions and ultrafine emulsions Halnor V.V. et al. [2018]. Nanoemulsions are stable and appeared as transparent and translucent due to small size droplet range Bhatt P. et al. [2011]. Nanoemulsion has been widely used in drug delivery, cosmetics Junyaprasert V.B. et al. [2009], and because of its small droplet size, long term stability and better solubilization capabilities. Ghosh PK. et al. [2006]. There is major difference between emulsion and nanoemulsion, emulsion is kinetically stable but thermodynamically unstable. nanoemulsion is very clear in physical appearance but emulsions are cloudy Halnor V.V. et al [2018].

Advantages of nanoemulsion

1. It gives site specific delivery of drugs Mangale M.R. et al. [2015].
2. It improves the bioavailability of drug.
3. It has improved physical stability of drug.
4. It facilitates drug to solubilize in its lipophilic vehicle Hussan R. et al. [2011].
5. It protects drugs from degradation with long term stability which leads to making an ideal drug delivery system Patil P.A. et al. [2016].
6. It is non-irritant and non-toxic.
7. It provides greater absorption because have smaller the droplet size droplets which having greater surface area Jaiswal M. et al [2015].

Disadvantages of nanoemulsion

1. Partial hydrolysis/ digestion of surfactant molecules by GIT lipase.
2. Over exaggerated use of large amount of surfactant and co-surfactant retarded its functionality Sharma N. et al. [2013].

Oral Administration of drug loaded nanoemulsion

Oral route is the easiest, most convenient and cost-effective way, for non-invasive drug administration Pinto J.F. et al. [2010]. This route is easy to achieving therapeutic targets due to increasing patient compliance for personalized

INTRODUCTION

Nanoemulsions are oil-in-water dispersions, having consists of oil, water, and S_{mix} (surfactant and co-surfactant mixture) of dispersion with size range of 20 to 200 nm. These are biphasic system in which one phase is disperse in the other phase, in the form in minute droplets Chime S.A. et al. [2014]. Nanoemulsion is biphasic dispersion, consists of oil phase, aqueous and higher proportion of surfactant and co-surfactant mixture of which yield size of nanoemulsion in the range of 50 to 200 nm. These systems possess intimately dispersed phase into another phase. These are two types of nanoemulsion such as oil/water (o/w) or water/oil (w/o) and bicontinuous Mason T.G. et al. [2006]. The dispersed phase is also known as internal phase or discontinuous phase, while the outer phase is called external phase or continuous phase. These are two types of nanoemulsion such as oil in water type (o/w) or water in oil type (w/o) and bicontinuous. It they appear transparent due to low size range Mishra R.K. et al. [2014]. It can also be defined as that nanoemulsions are thermodynamically stable, isotropically clear dispersion and stabilized by an interfacial film called as surfactant molecule. Also, it is known as miniemulsions, submicron emulsions and ultrafine emulsions Halnor V.V. et al. [2018]. Nanoemulsions are stable and appeared as transparent and translucent due to small size droplet range Bhatt P. et al. [2011]. Nanoemulsion has been widely used in drug delivery, cosmetics Junyaprasert V.B. et al. [2009], and because of its small droplet size, long term stability and better solubilization capabilities. Ghosh PK. et al. [2006]. There is major difference between emulsion and nanoemulsion, emulsion is kinetically stable but
compound with the chemical formula $C_{2n+3}$. Polyethylene Glycol (PEG) [2013].

increasing the solubilizing property of drug Zhou C et al. [2004]. They have limited ability to permeate the membrane and hygroscopic nature may present additional drawbacks Brüsewitz C et al. [2007]. There are too many approaches for increasing their bioavailability, including some approaches aremicronization, solid dispersion, complexation with cyclodextrins and use of particulate delivery systems, which are soluble/dispersible in aqueous phase Sachan et al. [2010]. By Carica papaya in Dengue-Carica papaya plant is popular or unlicensed herbal remedy is mainly used for the treatment of dengue, and it has been reported that Carica papaya have been increase the platelet count Rajapakse S. et al. [2019]. Carica papaya leaf has a various kind of benefits. In some parts of Asia, the young leaves of the papaya are steamed and eaten like spinach. It has been also stated that Papaya leaf juice helps increase white blood cells and platelets, normalizes clotting, and repairs the liver Aravind G. et al. [2013]. There are several plant species which are used to prevent the complications of thrombocytopenia but do not cure dengue but papaya plant is the only plant that are used treat dengue as well as thrombocytopenia.

**MATERIALS AND METHODS**

In order to design a dosage form for a drug, the basic need is to carry out preformulation studies on drug and its excipients. It is the essential stage, formulation is design which are intended with ideal drug release characteristics, optimal stability etc. The prime objective in preformulation studies, characterized the sample (drug) on the basis of its formulation excipients Bharate S.S. et al. [2013].

**Excipients profile:**

1. **Oleic Acid**- Oleic acid is a mono-unsaturated omega-9 fatty acids originate in various sources. Oleic acid is used for the preparation of nanoemulsion. It may be hinder the sequence of adrenoleukodystrophy, and it may be help in boost memory Moser B.R. et al. [2007].

2. **Tween 20**- Polysorbates are non-ionic surfactants which are use as emulsifying agents for the preparation of stable oil-in-water nanoemulsions. These are used for the increasing the solubilizing property of drug Zhou C. et al. [2013].

3. **Polyethylene Glycol (PEG)**- It is a polyether compound with the chemical formula $C_{2n}H_{4n+2}O_{n+1}$. It is slightly colorless viscous liquid and odorless. Polyethylene glycol basically used as co-solvent in nanoemulsion formulation Hutanu D. et al. [2014].

**Characterization & Identification of Drug (Carica papaya) & Excipients**

**Physical Appearance**: Green Powder

**Nature**: Non sticky, dry granular powder

**Odour**: Pleasant

**1.1 Spectrophotometry**

- UV spectrum of drug sample was measured through UV-Spectrophotometer (PerkinElmer, UV-160529).
- 10mg sample was weighed then it was transferred to 100 ml volumetric flask/diluted with methanol.
- After that, the stock solution was diluted up to 10 times, and UV spectrum was scanned in the range of 200-400nm.UV spectrum of the procured sample of Carica papaya powder showed a maximum absorption at maximum 267 nm which is identical with reported value in certificate of analysis Banala R.R. et al. [2015].

**Melting Point (MP) Determination**: Melting point of the drug sample was measured through melting point apparatus (Ambassador) using capillary method.

- A capillary tube was hold in hand and its one end was sealed.
- The open end of capillary tube was filled with dry drug powder.
- Then the capillary tube was entered in the melting point apparatus.
- The temperature, at which sample powder starts point to melt point was noted using thermometer and match with certificate of drug sample value or certificate of analysis of drug.
- It was observed the melting point of Carica papaya powder sample was found to be 89°C.
- It is matched to the reported melting point of Carica papaya powder.

**1.4 Determination of drug solubility**

- The solubility of the drug in various excipients like surfactant, co-surfactant, oil phase and Smix (mixture of surfactant and co-solvent)
- It was determined by dissolving the excess amount of drug taken in eppendorf tube (1.0 mL) containing excipient.
- If the drug gets dissolved, then again, an excess amount of the drug was added in each 2mL eppendorf tube and mixed with the help of vortex mixer.
- The eppendorf tube was then kept at 25 ± 5°C for 72 hrs to get to equilibrium attained.
- After 72 hrs, drug samples were centrifuged at the 3000rpm for 10min using instrumentation model no, make.
Table 1: Solubility of *Carica papaya* powder in oils, surfactant and co-surfactants

<table>
<thead>
<tr>
<th>Oil</th>
<th>Surfactant</th>
<th>Co-surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almond oil</td>
<td>Tween 80</td>
<td>Propylene Glycol (PG)</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>Tween 20</td>
<td>Polyethylene Glycol 200</td>
</tr>
<tr>
<td>Olive oil</td>
<td>Span 80</td>
<td>Polyethylene Glycol 400</td>
</tr>
<tr>
<td>Castor oil</td>
<td>Span 20</td>
<td>Transutol IP</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>Solutol HS-15</td>
<td>Propylene Glycol (PG)</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>Tween 20</td>
<td>Polyethylene Glycol 400</td>
</tr>
</tbody>
</table>

Table 2: Summary of different trial batches

<table>
<thead>
<tr>
<th>Trials</th>
<th>Smix components</th>
<th>Smix ratio</th>
<th>Oil type</th>
<th>Water (mg)</th>
<th>Visual Exam.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tween 20/ PEG 400</td>
<td>1:1</td>
<td>Almond oil</td>
<td>Infinite</td>
<td>Turbid</td>
</tr>
<tr>
<td>2</td>
<td>Tween 20/ PEG 400</td>
<td>1:1</td>
<td>Olive oil</td>
<td>Infinite</td>
<td>Clear</td>
</tr>
<tr>
<td>3</td>
<td>Tween 20/ PEG 400</td>
<td>1:1</td>
<td>Castor oil</td>
<td>Infinite</td>
<td>Turbid</td>
</tr>
<tr>
<td>4</td>
<td>Tween 20/ PEG 400</td>
<td>1:1</td>
<td>Soybean oil</td>
<td>Infinite</td>
<td>Turbid</td>
</tr>
<tr>
<td>5</td>
<td>Tween 20/ PEG 400</td>
<td>1:1</td>
<td>Sesame oil</td>
<td>Infinite</td>
<td>Turbid</td>
</tr>
<tr>
<td>6</td>
<td>Tween 80/ PG</td>
<td>1:1</td>
<td>Oleic acid</td>
<td>Infinite</td>
<td>Turbid</td>
</tr>
<tr>
<td>7</td>
<td>Tween 80/ PEG 400</td>
<td>1:1</td>
<td>Oleic acid</td>
<td>Infinite</td>
<td>Turbid</td>
</tr>
<tr>
<td>8</td>
<td>Tween 20/ PEG 400</td>
<td>1:1</td>
<td>Oleic acid</td>
<td>Infinite</td>
<td>Clear</td>
</tr>
<tr>
<td>9</td>
<td>Tween 20/ PEG 400</td>
<td>1.5:1</td>
<td>Oleic acid</td>
<td>Infinite</td>
<td>Clear</td>
</tr>
<tr>
<td>10</td>
<td>Tween 20/ PEG 400</td>
<td>1:1.5</td>
<td>Oleic acid</td>
<td>Infinite</td>
<td>Clear</td>
</tr>
<tr>
<td>11</td>
<td>Tween 20/ PEG 400</td>
<td>2:1</td>
<td>Oleic acid</td>
<td>Infinite</td>
<td>Clear</td>
</tr>
</tbody>
</table>

Systems containing Span 80 or Span 20 yielded turbid systems

Table 3: Ternary phase diagram drawn at (1:1) Smix ratio, oleic acid and water

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Oil (mg)</th>
<th>Smix (mg)</th>
<th>Water (mg)</th>
<th>Oil %</th>
<th>Smix %</th>
<th>Water %</th>
<th>Visual examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>90</td>
<td>55</td>
<td>6.45</td>
<td>58.06</td>
<td>35.49</td>
<td>Clear</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>100</td>
<td>480</td>
<td>3.33</td>
<td>16.67</td>
<td>80.00</td>
<td>Clear</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>80</td>
<td>65</td>
<td>12.12</td>
<td>48.48</td>
<td>39.40</td>
<td>Clear</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>160</td>
<td>97</td>
<td>7.22</td>
<td>57.76</td>
<td>35.02</td>
<td>Clear</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>120</td>
<td>60</td>
<td>10.00</td>
<td>60.00</td>
<td>30.00</td>
<td>Clear</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>70</td>
<td>185</td>
<td>10.53</td>
<td>24.56</td>
<td>64.91</td>
<td>Clear</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>60</td>
<td>65</td>
<td>24.24</td>
<td>36.36</td>
<td>39.40</td>
<td>Clear</td>
</tr>
</tbody>
</table>

Table 4: Design and composition of the nanoemulsion formulations showing various components

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Oil (mg)</th>
<th>Smix (mg)</th>
<th>Fixed amount of <em>Carica papaya</em> Leaf powder (mg)</th>
<th>Total weight* (mg)</th>
<th>Fixed amount of water (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 (20:80)</td>
<td>26.8</td>
<td>107.2</td>
<td>10</td>
<td>144</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Smix = Tween20/PEG 400 (2:1); oil phase (Oleic acid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2 (20:80)</td>
<td>26.8</td>
<td>107.2</td>
<td>10</td>
<td>144</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Smix = Tween20/PEG 400 (1.5:1); oil phase (Oleic acid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3 (10:90)</td>
<td>13.4</td>
<td>120.6</td>
<td>10</td>
<td>144</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Smix = Tween20/PEG 400 (1:1); oil phase (Oleic acid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F4 (20:80)</td>
<td>26.8</td>
<td>107.2</td>
<td>10</td>
<td>144</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Smix = Tween20/PEG 400 (2:1); oil phase (Oleic acid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Droplet size and PDI of the formulation (Zeta sizer)

<table>
<thead>
<tr>
<th>Formulations</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Droplet size (nm)</td>
<td>70.36</td>
<td>82.38</td>
<td>80.63</td>
<td>63.37</td>
</tr>
<tr>
<td>PDI</td>
<td>0.128</td>
<td>0.160</td>
<td>0.125</td>
<td>0.187</td>
</tr>
</tbody>
</table>

- The supernatant was taken and filtered using 0.45µm membrane filter. And suitable diluted with methanol.
- The amount of drug dissolved in each excipient was measured through UV-spectrophotometer (PerkinElmer, UV-160529) at their respective λ<sub>max</sub> using the following formula Zeng L. et al. [2016].

Solubility (µg/ml) = (Absorbance± intercept)/slope × Dilution Factor
1.5 Aqueous Dispersibility of pre-concentrate mix: Various components namely oil, surfactant, and co-surfactant are required for the designing of nanoemulsion and its dilution behavior in the presence of aqueous media. Aqueous Dispersibility of nanoemulsion was assessed through visual examination upon gradual addition of aqueous phase in the pre-concentrate mix. Dilatation of the pre-concentrate mixture with aqueous phase could produce a clear system or turbid dispersion indicates formation of nanoemulsion or coarse emulsion respectively. The quantity of water is determined after visual inspection; to make the pre-concentrate mix remained in transparent containers D.G. Fatouros et al. [2007].

- 5mL each of tween 20 and polyethylene glycol was transferred to 15 mL capacity falcon tube mix well for 20 min gradually. It is labeled as Smix (1:1) containing surfactant and co-surfactant fractions in equal proportions.
- 100μL of oleic acid was taken in 2mL eppendrof tube as an oil phase and to it, 900μL of Smix (prepared on step 1) was transferred and mix through vortex shaker.
- Subsequent additions of 100μL of water were made to the above eppendrof tube containing oil phase and Smix.
- Initially 100μL of water was added and shake with the help of vortex well till clear solution was observed.
- Then, 1mL of formulation was taken in 10mL tube containing oil phase and Smix.
- The content of each beaker was sonicated using the instrument model make.
- Then the collected sample was sonicated for 10 minutes.
- Aqueous phase was added in the drug loaded discontinuous phase in drop wise manner under continuous stirring and sonication.

3. Evaluation of Carica papaya Nanoemulsions

3.1 Physical characterization of Carica papaya Nanoemulsion Formulation

3.1.1 Droplet size distribution
- Droplet size of different formulations was measured through Malvern, zeta sizer.
- 5mL of sample was taken and filtered through nylon membrane filter.
- Then the collected sample was sonicated for 10 minutes.
- Data was represented as percentage of droplet intensity or percentage of volume v/s the droplet size of nanoemulsion system. Shen J. et al. [2016].

3.2 Measurement of Electrical Conductivity of Carica papaya Nanoemulsion Formulation
- Evaluation of electrical conductivity of the Carica papaya nanoemulsion formulation was determined using digital conductometer meter model 611 E.
- First, check the cell constant of the instrument using the standard solution of KCl at 25°C.
- Then the collected sample was sonicated for 10 minutes.
- Conductance value displayed on the conductometer was recorded until a constant value was registered by instrument.
- Each formulation was further gradually diluted with aqueous phase (in the fraction of 50μL of distilled water) to the beaker.
- Conductance of dispersion system was recorded as per the procedure given above in 5.22 Daar J. et al. [2017].

3.3 Determination of RI (Refractive Index)
- Refractive index was determined with the help of refractometer.
Formulation and evaluation of Carica papaya nanoemulsion for treatment of dengue and thrombocytopenia

Table 6: Variations in the electrical conductivity values of the formulations incorporating aqueous phase dilutions

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Addition of water (µL)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.9</td>
<td>0.8</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0.6</td>
<td>1.5</td>
<td>0.9</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>1.9</td>
<td>2.8</td>
<td>1.9</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>1.4</td>
<td>2.3</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Table 7: Refractive index of formulations with addition of aqueous phase dilution

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Water addition (µL)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>1.448</td>
<td>1.452</td>
<td>1.445</td>
<td>1.442</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>1.455</td>
<td>1.453</td>
<td>1.450</td>
<td>1.437</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>1.454</td>
<td>1.455</td>
<td>1.458</td>
<td>1.449</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>1.455</td>
<td>1.456</td>
<td>1.456</td>
<td>1.448</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>1.450</td>
<td>1.453</td>
<td>1.451</td>
<td>1.447</td>
</tr>
</tbody>
</table>

Figure 1: Representation of UV spectrum of Carica papaya powder in methanol (sample)

Figure 2: Ternary phase drawn at (1:1) Smix ratio, oleic acid and water

Figure 3: Droplet size and its distribution measured from zeta sizer

Figure 4: FTIR spectrum of formulation-4 containing oil, Smix and 10µL water

Figure 5: FTIR spectrum of formulation-4 containing oil, Smix and 20µL water
To each preconcentrate formulation (1mL), five different equal proportions (each 100μL) were taken in a 10ml capacity beaker.

Add distilled water of each five beaker at ratio 50, 100, 150, 200 and 250μL and mix thoroughly for 30 min.

ABBE Refractometer was kept at appropriate position to get sufficient light.

The refractometer was kept thoroughly, and clean using methanol and mirror assembly was aligned in a way to get light over its eye pieces.

Single drops were taken and apply from the system whose refractive index need to be determined, was placed over the stage assembly of refractometer

Keep its assembly closure shut down so that the entire system would uniformly spread the liquid over its prisms.

Adjust the knobs, so, that the cross mark was visually appeared and then particular value was recorded.

The same procedure was followed to determine the refractive index of drug loaded formulations Pangeni R. et al. [2014].

3.4 Aqueous dilution behaviour of formulation using FTIR

Aqueous dilution behaviour of each formulation was studied using FTIR spectroscopy.

Each pre-concentrate mix formulation weighing 100μl was taken in 5mL capacity beakers.

And diluted with 10 and 20μl with distilled water respectively and mixed uniformly.

Diluted nanoemulsion formulations were taken for FTIR spectroscopy using Shimadzu instrumentation by KBr pellet method.

Different spectra of placebo formulations (Without API) were also recorded and subject to identification of –OH group frequency analysis Nasr A. et al. [2016].

3.5 pH determination

Before the start of pH determination, pH meter was calibrated with standard buffer solutions. Therefore, the pH of the formulation was previously adjusted to 6.5-7.2.

The pH of the Carica papaya formulations was measured by a digital pH meter make Symtronic; model SE 962 P. pH of the Carica papaya formulations is a parameter to be required to avoid intestinal irritation Mahajan H. et al. [2017].

3.6 Stability studies for optimized formulations

The stability studies of Carica papaya nanoemulsion were performed for three months using stability chamber make (Mac, model CAT No MSW-127).

The optimized formulation was accurately measure.

Filled in the amber color bottle then wrapped in black paper.

Stored in the zipper bag and placed in the humidity chamber.

The temperature and humidity condition of the chamber was maintained at 25 ± 1°C, 25 ± 1°C, and 40 ± 1°C.

Each formulation was stored for the periods of three months.

The formulations were analyzed at the end of 0, 1, 2, and 3 months Lei Y. et al. [2013].

The sample formulation was analyzed for physical appearance and viscosity of the formulation.

3.7 Morphological evaluation of formulation

The morphological and structural evaluation of the formulations was carried out with the help of transmission electron microscopy (TEM) Mahajan H. et al. [2017].

From undiluted samples, droplet was kept over wax coated paper.

Then placing a circular grid, in the film form which is made up of copper metal, with size 400 mesh was externally applied.

Sample was stained with 10uL of 2% (w/v) phosphotungstic acid for few seconds (approx. 10sec) and kept the sample dried.

Surface morphology of sample was recorded on the instrumentation model Morgagni 268D transmission electron microscopy.
electron microscopes operated at 70kV Pandey Y.R. et al. [2015].

RESULT AND DISCUSSIONS

It has been reported that Carica papaya has play a beneficial role and having many kinds of nutrition’s which are used for different kind of treatment because it has many kinds of chemical properties. It has also been stated that Carica papaya has been promising prospective against the treatment of dengue disease. The aim of this work is to design a Carica papaya based nanoemulsion intended for oral use. The aim of present work is to explore the new formulation of Carica papaya based nanoemulsion to enhance the bioavailability through oral route and also represent the new drug discovery for better treatment of dengue disease as well as thrombocytopenia. Owing to nano droplet size of nanoemulsion, better drug solubilization characteristics in presence of its formulation components viz. surfactant and co-solvent.

In the preformulation studies of this work, primarily identification of procured drug sample and excipients. There are many methodologies used for the characterization of Carica papaya leaf. Organoleptic of the characterization of Carica papaya leaf powder (drug sample) showed that the procured sample was found to be existed in Green powder, with pleasant odour. Spectrophotometry of drug sample was measured in methanol is given in figure-1. Results that, sample has $\lambda_{\text{max}}$ value at 267 nm which is almost identical to that of spectrum. An analytical methodology is used for the measurement of U.V. spectrum of drug sample. This methodology is used to quantify the amount of Carica papaya drug sample in solution.

Drug solubility is most essential tool to develop a nanoemulsion formulation where we find out its miscibility with liquid excipients, such as drug solubility in oil phase, surfactant and co-surfactant. Different excipients to be required for Carica papaya leaf powder solubility in the formulation development stage, combinations are given in table no-1. Highest solubility is fund to be Oleic acid, tween 20 and polyethylene glycol 400 respectively in ratio 54:48:45 mg/mL.

In another study, for the characterization of aqueous dispersibility of different excipients in which observe the either formation of clear dispersion or formation of coarse emulsion. All the observation has been based on assumption so that nanoemulsion upon aqueous dilution to give clear dispersion system. Phase behaviour resulted from drug loading nanoemulsion excipients to give clear dispersion was also evaluated.

To explore the dilution of pre-concentrate, several trials were tried. There are total 11 trials were done to explore the preconcentrate dilution. Overall summary of various trials conducted at pre-formulation stage, were concluded that in order to find out the possible ternary component used for nanoemulsion systems are given in the table 2. So, the result was, trail 8, 9, 10 and 11 were the possible ternary component and these combinations would be taken up for the development of nanoemulsion system of Carica papaya.

The mapping tool also called as ternary phase diagram, gives us a plot which are very helpful in the preformulation studies on nanoemulsion. Therefore, it is important for possible dispersion system, were formed when a particular proportion of $S_{\text{mix}}$ oil and water phase taken. To sum up the phase diagram studies, region showing nanoemulsion area is increased from $S_{\text{mix}}$ ratio 1:1. Phase diagrams (figure-2) prepared at Smix ratios 1:1 is only considered into account for the development of nanoemulsion formulation.

Formulation of Carica papaya based nanoemulsion having different kind of composition which contains oil, Smix, drug and aqueous phase is given in the table 4. All 4 formulation are designed according to the plotted phase diagram which are prepared at Smix level 1:1 with fixed amount of Carica papaya and aqueous water respectively. All the formulations were evaluated for physical characters viz. droplet size, conductivity, refractive index etc.

1. Droplet size and its polydispersity index- It was determined with the help of Malvern zeta sizer. All the formulation (F1 to F4) have different size range 70.36, 82.38, 80.63 and 63.37 nm and results are recorded in table 5. Meanwhile the droplet size of formulation is in good arrangement to the definition of nanoemulsion (50-200 nm) with good polydispersity index indicates uniformity of droplets size.

2. Measurement of Electrical Conductivity of Carica papaya Nanoemulsion Formulation- This examination is used for find out how the various microstructures are formed and it’s rearranged from oil continuous to water continuous domains, upon the aqueous dilution to pre-concentrate mix. Primarily the conductivity had lower because of formulation of oil continuous microstructure domain so in other words, it could lead to w/o types of nanoemulsion. The main aim was to determine the micro structural changes and it remains in w/o or o/w systems. All the value of formulation F1, F2, F3 and F4 are given in the table 6. Initially, all these measurements (shown in table 6) of electrical conductivity are existed in w/o type nanoemulsion but changed in bicontinuous system upon gradual addition of aqueous phase up to 250mg. It has been observed that the conductivity of formulation no F2 and F4 shown least changes when incorporation of 100mg of aqueous phase and have been remained in w/o nanoemulsion systems. Although, the formulation no F1 and F3 transformed to bicontinuous systems when added in same quantity of water. Since it has been evaluated that the electrical conductivity of the formulation was gradually increased with aqueous phase volume. If the subsequent addition of water (volume lower then 100mg) to the oil continuous phase then resulted as breakage of nanoemulsion and transformed to bi-continuous domains were formed. Fall in the conductivity value come to a constant.
which indicates water continuous domain is formed and hence o/w nanoemulsion was formed in all formulation. Finally, extra amount of water (beyond 250mg) leads to cosarsening of formulations.

3. Determination of Refractive Index of Carica papaya Nanoemulsion Formulation - ABBE refractometer was used for the determination of RI (refractive index). Refractive index of all formulations F1, F2, F3 and F4 are respectively given in table 7. Upon addition of water phase to formulations resulted micro-structural transitions similar to that of electrical conductivity.

4. Aqueous dilution behavior of Carica papaya Nanoemulsion formulation using FTIR Spectrophotometry- All the FTIR spectra of the formulation formulations is given in the figures 4 and 5. To determine the nature of water phase entrapped in the nano droplets of formulations, selectively extra amount of aqueous phase was incorporated 10-20uL in the formulations as well as in placebo. Water possesses –OH group and its vibrational frequency appeared at 3400 cm-1 and placebo showed vibrational frequencies from 3205 to 3491cm-1 with lowest transmittance values. Conclusion of these results indicate that, as aqueous phase fraction increases then its frequencies will also increase to larger values which in turn resulted due to larger exposure of water droplet to radiation.

5. pHdetermination- The pH of the formulations was found to be in the range of 6.7. As we know that, ingredients are non-ionic (surfactant) and inert (solvent) that are used for the formulation. So, all selected formulation pH nearby intestinal fluid pH range.

6. Stability studies for optimized formulations- This examination for the Carica papaya nanoemulsion where stability was checked out at room temperature. It was studied at 40 ± 1°C and 70 ± 5% RH for three-month duration as per the ICH guidelines. It was also observed that, formulations (F1, F2, F3 and F4) were viscous oily odorless, transparent and of thick liquid consistency. It was also identified that, changes of colour green (initial month) to light green (after three month) was observed.

7. Morphological evaluation of the Formulation- Morphological examination of the formulation was done through the transmission electron microscopy (TEM). All the morphological features were explored, as given in the figure 6. Resulted as F4 formulation showed a batter droplet size in the size range of 63.37nm.

CONCLUSION
Carica papaya leaf powder (drug) and all excipients were authenticated using UV, FTIR spectrophotometry for the preformulation studies of nanoemulsion design. Drug excipient compatibility studies were performed in order to rule out any possibility of interactions. Drug concentration was established by the analytical methodology (spectrophotometric method). Then various trails were performed to screen out the excipients which could form the possible surfactant, co-surfactant and oil phase of nanoemulsion formulation of Carica papaya. The selection of all excipients was investigated on basis of mutual miscibility, aqueous dispersibility and solubility of drug in the selected nanoemulsion components, then the ternary phase diagram was drowned to produce nanoemulsion of Carica papaya. Various formulations were prepared selectively F1, F2, F3 and F4 with the help of ternary phase diagram and finally all the formulations were evaluated by the help of different evaluation parameter such as conductivity, refractive index, droplet size distribution. FTIR study was also done to reveal its localization in nanoemulsion formulation upon aqueous dilution. Finally, it was observed that optimum nanoemulsion formulation consisted of Snw (Tween 20/Polyethylene glycol 400) and oil phase (Oleic acid) and Carica papaya leaf powder (drug) according to the particle size range (63.37nm).

Future Scope
Basically, this formulation of Carica papaya nanoemulsion was prepared for the treatment of dengue disease as well as thrombocytopenia. Nanoemulsion formulation of Carica papaya was not discovered yet, so this formulation will help improve the oral bioavailability in dengue patient. Carica papaya leaf having ability to inhibits the dengue chain. According to the conclusion, various formulations were prepared selectively F1, F2, F3 and F4 with the help of ternary phase diagram, So, the formulation F4 was shown great response according to the particle size range (63.37nm), as per the evaluation parameters. After evaluation of this formulation, we will be able develop the better dosage form of nanoemulsion. And other evaluation parameters should be exploring for well understand about the formulation therefore the future scope this formulation, will help to tackle the nemesis of dengue disease.

CONFLICT OF INTEREST
The author has declared that no conflicts of interest exist.

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