DESIGN, DEVELOPMENT AND EVALUATION OF CURCUMIN LIPOSOMES

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ABSTRACT
The purpose of this investigation was to design, develop and to evaluate the liposomal drug delivery system for curcumin, an herbal drug isolated from Curcuma longa. Liposomes were prepared by using phospholipid as carrier. Eight formulations (CL1 – CL8) were designed and prepared by varying the concentrations of phospholipids. All the prepared formulations were evaluated. The results showed that CL4 formulation was found to be best amongst other formulations.

Key words: Curcumin, Curcuma longa, liposomes, phospholipid, bioavailability.

1. INTRODUCTION
Curcumin, an herbal drug isolated from Curcuma longa family zingiberaceae, is used in the treatment of cancer, alzheimer and other inflammatory conditions.¹²³ The chemical structure of curcumin (IUPAC name - (1E,6E)-1,7-bis (4-hydroxy- 3-methoxyphenyl) -1,6- heptadiene-3,5-dione) is given below
The drug is soluble in oil and insoluble in water at acidic and neutral pH. But the solubility increases when the medium becomes alkaline. The bioavailability of the drug is very poor and it is in very low level in plasma and tissues. Hence there is a demand to increase the bioavailability of curcumin before administering to the patients. Lot of ideal ways and drug delivery systems are available for increasing the bioavailability. Based on the drug character, liposomal approach seems to be good when comparing to other approaches.

Liposomes are the spherical shaped small vesicles that can be produced from cholesterols, non-toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins. Phospholipids spontaneously form a closed structure when dissolved in water with internal aqueous environment bounded by phospholipids bilayer membranes, this vesicular system is called as liposome. Liposomes are the drug carrier loaded with different variety of molecules such as small drug molecules, proteins, nucleotides and even plasmids. Liposomes mainly consist of phospholipids and cholesterol as its structural components.

In this present study it was planned to prepare curcumin as liposomes by using phospholipids to enhance its bioavailability.

2. MATERIALS AND METHODS

2.1. Materials

Curcumin – in-house preparation (extraction from Curcuma longa – Pharmacognostical lab, SPS, Vels University), phospholipid from lipoid, Germany, eudragit S100 from Lobachemie Pvt, Ltd, Mumbai, other chemicals and solvents used were of analytical grade.

2.2. Methods

2.2.1. Preformulation studies

Preformulation testing is the initial step in formulation of a drug to various dosage forms. It is a process of choosing the suitability of the drug delivery by determining the physicochemical properties of a new compound which can affect the drug performance and development of a highly efficacious and safe drug and its dosage form. It gives the information needed to define the nature of the drug substance and provide a framework for the drug combination
with pharmaceutical excipients in the dosage form. Hence, Preformulation studies were
performed to confirm the absence of interaction of drug with other additives. The
preformulation studies carried out for the present work were;

- Compatibility studies – By FTIR and DSC
- Standard curve of Curcumin

2.2.1.1. Fourier Transform Infra Red spectroscopy
IR spectra of curcumin and other excipients used in the formulation were recorded by using
“Perkin-Elmer FTIR.” The sample for the IR spectroscopy was prepared by mixing the
samples with spectroscopic grade KBr and compressed into transparent pellets, then scanned
in the IR range from 500 to 4000 cm$^{-1}$ with a resolution of 4 cm.$^7$ The results were given in
results and discussion section.

2.2.1.2. Differential Scanning calorimetry
Differential Scanning Calorimetry studies were carried out using “Schimadzu DSC-60.$^7$ In
this study curcumin was mixed with the excipients used in the formulation and thermal
analysis of each sample was carried out. During the study, the temperature range from 25 to
600º C, heating rate 10ºC/min and flow rate of nitrogen 30 ml/min were maintained.
Approximately 5mg of samples were taken in aluminum pan sealed and the thermogram was
recorded.
The results were given in results and discussion section.

2.2.1.3. Standard graph of Curcumin
Standard graphs of the drug were prepared by dissolving different concentrations of standard
curcumin in pH 7.4 phosphate buffer. The absorbance was measured at 425nm. Linear
relationship was observed with absorption to concentration of drug. The values of absorbance
related to concentration were given in results and discussion section.

2.2.2. Formulation of Curcumin Liposomes$^8$
Method of preparation
Step1: Preparation of Curcumin liposomes
Specified concentration of phospholipid was dissolved in sufficient quantity of chloroform
and it was spread as a thick film on the inner wall of a round bottomed flask. The flask was
kept in a vacuum to remove the solvent completely. Curcumin was dissolved in pH 7.4
phosphate buffer solution separately and it was added to the thick lipid film in the round bottom flask to disperse the lipid (reverse rotation of flask).

The above suspension containing curcumin liposome was suspended in 10 ml of sodium alginate solution and mixed completely.

**Step2: Calcium alginate gel beads loaded with curcumin liposomes**

The suspension of curcumin liposome was dropped into about 30 ml calcium chloride solution with mild agitation and stirred slowly for 1 h, and kept for 6 h to get calcium alginate gel beads.

**Step3: Eudragit S100-calcium alginate gel beads loaded with curcumin liposomes**

The wet calcium alginate beads were transferred into 2% Eudragit S100 solution and subjected to gentle magnetic stirring for 30 minutes. The resulting alginate -eudragit S100 beads were collected and rinsed with deionized water and air dried for overnight. Then the final product was dried in a vacuum for 12 h.

All the formulations (CL1-CL8) were prepared in the similar method by varying the concentrations of phospholipid.

Formula used for the preparation of curcumin loaded liposomes were described in Table 1

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin (mg)</td>
<td>CL1 CL2 CL3 CL4 CL5 CL6 CL7 CL8</td>
</tr>
<tr>
<td>Phospholipid (mg)</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>50 75 100 125 150 175 200</td>
</tr>
<tr>
<td>Chloroform</td>
<td>q.s. q.s. q.s. q.s. q.s. q.s. q.s.</td>
</tr>
<tr>
<td>Sodium alginate solution (ml)</td>
<td>10 10 10 10 10 10 10 10</td>
</tr>
<tr>
<td>Calcium chloride solution(ml)</td>
<td>30 30 30 30 30 30 30 30</td>
</tr>
<tr>
<td>Eudragit S100 (%)</td>
<td>2 2 2 2 2 2 2 2</td>
</tr>
</tbody>
</table>

**2.2.3. Evaluation of curcumin liposomes**

**2.2.3.1. Particle size and zeta potential**

The prepared liposomes were evaluated for their particle size and zeta potential by photon correlation spectroscopy (PCS) using Zetasizer. The formulations were diluted to 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per second (kcps). Analysis was carried out at 25°C with an angle of detection of 90°.
2.2.3.2. Liposome Morphology

Transmission Electron Microscopy (TEM) was used to study the curcumin liposome’s morphology. TEM studies were performed at 8 kV. The copper grid was fixed into sample holder and placed in vacuum chamber of the transmission electron microscope and observed under low vacuum and TEM images for recorded. The results were given in results and discussion section.

2.2.3.3. Encapsulation efficiency

Separation of unentrapped drug from the prepared liposomes was carried out and analyzed using UV Visible spectrophotometer at a λmax 425nm. Liposomes prepared without drug were treated in similar manner and served as blank for the above study. The formula used to calculate encapsulation efficiency was given below

\[
\text{Encapsulation efficiency} = \frac{\text{Entrapped drug (mg)}}{\text{Total amount of drug added (mg)}} \times 100
\]

2.2.3.4. In vitro drug release studies

In vitro release studies were performed using dialysis membrane method. The prepared liposomal formulation was placed inside a dialysis membrane immersed in aqueous buffer of volume 100 ml (Phosphate buffer pH 7.4). At predetermined time intervals the sample was withdrawn and the amount of curcumin was determined by measuring the absorbance at 425nm using a UV-Visible spectrophotometer. From the absorbance values the cumulative percentage drug release was calculated.

3. RESULTS AND DISCUSSION

3.1. Preformulation studies

3.1.1. Compatibility study using IR and DSC

IR spectrum of curcumin standard consisted of characteristics band values. These characteristic band values were observed in all the recorded IR spectra for the isolated curcumin sample and curcumin-excipient mixtures.

DSC of curcumin showed a sharp endothermic peak at 178°C (melting point). The physical mixture of curcumin with other excipients also showed the same thermal behaviour as the individual component. DSC results also revealed that the physical mixture of curcumin with
Excipients showed superimposition of the thermogram. There was no significant change observed in melting endotherm of physical mixture of curcumin and excipients. Hence from the IR and DSC studies, it was found that there was no interaction took place between curcumin and other ingredients used in the formulation. From the IR and DSC reports it was concluded that the curcumin was found to be compatible with other excipients used in the formulation.

The FTIR and DSC spectra were given in the Fig. 2 to 5.

![Fig 2: FT-IR Spectrum of curcumin](image1.png)

![Fig. 3 FT-IR Spectrum of curcumin liposomes](image2.png)
3.1.2. Standard curve of curcumin

Table 2 Standard curve of curcumin

<table>
<thead>
<tr>
<th>S.no</th>
<th>Concentration(µg/ml)</th>
<th>Absorbance(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>10</td>
<td>0.012</td>
</tr>
<tr>
<td>3.</td>
<td>20</td>
<td>0.023</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>0.035</td>
</tr>
<tr>
<td>5.</td>
<td>40</td>
<td>0.047</td>
</tr>
<tr>
<td>6.</td>
<td>50</td>
<td>0.058</td>
</tr>
</tbody>
</table>
3.2. Curcumin liposomes – formulation and evaluation

Curcumin liposomes were successfully prepared and evaluated. The appearance of the curcumin liposomes (CL4) was given in fig.7.

Fig. 7 Photographs of Curcumin liposomes (CL4)

Eight formulations (CL1-CL8) were prepared by taking different drug to lipid ratios. The optimum concentration of phospholipid was selected based on its encapsulation efficiency and \textit{in vitro} drug release profile.

3.2.1. Zeta potential, Particle size and Entrapment efficiency

The results of zeta potential, particle size and entrapment efficiencies were given in table 3 and figs. 8 and 9.
Table 3 Zeta potential, Particle size and entrapment efficiency of CL1-CL8

<table>
<thead>
<tr>
<th>Trials</th>
<th>Zeta potential (mV)</th>
<th>Particle size(nm)</th>
<th>Entrapment Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL1</td>
<td>-19.6±3.8</td>
<td>210.24±8.32</td>
<td>60.69±0.21</td>
</tr>
<tr>
<td>CL2</td>
<td>-17.8±2.6</td>
<td>220.66±10.18</td>
<td>68.53±0.18</td>
</tr>
<tr>
<td>CL3</td>
<td>-17.6±3.2</td>
<td>225.78±9.31</td>
<td>76.78±0.80</td>
</tr>
<tr>
<td>CL4</td>
<td>-16.6±2.8</td>
<td>245.82±3.26</td>
<td>90.63±0.29</td>
</tr>
<tr>
<td>CL5</td>
<td>-15.4±3.8</td>
<td>325.02±6.26</td>
<td>90.39±0.11</td>
</tr>
<tr>
<td>CL6</td>
<td>-14.6±2.4</td>
<td>340.12±12.18</td>
<td>90.66±0.21</td>
</tr>
<tr>
<td>CL7</td>
<td>-15.6±2.6</td>
<td>375.56±9.31</td>
<td>90.74±0.08</td>
</tr>
<tr>
<td>CL8</td>
<td>-15.4±2.8</td>
<td>500.12±9.27</td>
<td>90.67±0.28</td>
</tr>
</tbody>
</table>

mean±S.D, n=3

Particle size and entrapment efficiency of the curcumin liposomes (CL1-CL4) were increased with increasing the lipid content up to 100 mg. This may be due to high amount of availability of lipid to encapsulate the drug, upon increasing the lipid content, number of layers coated the drug was increased, this resulted in increased particle size and entrapment efficiency. Further increase in the lipid concentration to 125 mg as in (CL5-CL8), there is no much increase in the entrapment efficiency due to the availability of the drug to be incorporated is low which is not enough for further encapsulation of drug by lipid.

Fig 8 Zeta potential of curcumin liposomes (CL4)
3.2.2. Liposome Morphology

According to morphological evaluation analysis by transmission emission microscopy, all the prepared curcumin liposomes (CL1-CL8) seemed to have a similar spherical shape. The sizes of all the formulations were in nano meter.

The TEM photograph of the formulation CL4 was given in fig.10

Fig. 10.Curcumin loaded liposomes (CL4)
### 3.2.3. In vitro release

The results of in vitro release were given in table 4.

#### Table 4 Percentage In vitro drug release of CL1-CL8

<table>
<thead>
<tr>
<th>Trials/Time (hrs)</th>
<th>CL1</th>
<th>CL2</th>
<th>CL3</th>
<th>CL4</th>
<th>CL5</th>
<th>CL6</th>
<th>CL7</th>
<th>CL8</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>34.55±0.53</td>
<td>24.66±0.60</td>
<td>20.30±0.92</td>
<td>8.12±0.51</td>
<td>2.32±0.10</td>
<td>2.19±0.15</td>
<td>1.63±0.55</td>
<td>1.62±0.00</td>
</tr>
<tr>
<td>1</td>
<td>43.04±0.61</td>
<td>33.24±0.50</td>
<td>30.20±1.03</td>
<td>20.16±0.19</td>
<td>8.62±0.00</td>
<td>6.65±0.57</td>
<td>5.72±0.09</td>
<td>5.32±0.00</td>
</tr>
<tr>
<td>1.5</td>
<td>46.28±0.58</td>
<td>39.66±0.60</td>
<td>33.82±0.54</td>
<td>29.20±0.91</td>
<td>12.83±0.88</td>
<td>8.17±0.41</td>
<td>6.85±0.39</td>
<td>6.17±0.32</td>
</tr>
<tr>
<td>2</td>
<td>55.54±1.02</td>
<td>46.30±0.98</td>
<td>43.45±0.08</td>
<td>34.80±0.12</td>
<td>19.69±0.64</td>
<td>13.42±0.85</td>
<td>9.09±0.38</td>
<td>8.32±0.11</td>
</tr>
<tr>
<td>2.5</td>
<td>62.77±0.11</td>
<td>55.72±0.09</td>
<td>49.28±0.96</td>
<td>39.12±0.71</td>
<td>25.38±1.29</td>
<td>20.20±0.10</td>
<td>16.14±0.65</td>
<td>13.22±0.00</td>
</tr>
<tr>
<td>3</td>
<td>68.66±0.00</td>
<td>62.15±0.43</td>
<td>55.94±0.61</td>
<td>39.86±0.65</td>
<td>28.12±0.38</td>
<td>24.84±0.55</td>
<td>20.48±0.52</td>
<td>17.53±0.30</td>
</tr>
<tr>
<td>4</td>
<td>73.86±0.48</td>
<td>69.60±0.00</td>
<td>61.29±1.20</td>
<td>42.20±0.36</td>
<td>32.14±0.25</td>
<td>29.10±0.67</td>
<td>25.40±0.39</td>
<td>21.42±1.22</td>
</tr>
<tr>
<td>6</td>
<td>85.26±1.00</td>
<td>73.30±0.17</td>
<td>69.11±0.78</td>
<td>44.78±0.20</td>
<td>33.76±0.76</td>
<td>32.02±0.85</td>
<td>29.65±0.57</td>
<td>25.02±0.58</td>
</tr>
<tr>
<td>8</td>
<td>90.31±0.98</td>
<td>78.27±1.11</td>
<td>73.25±1.15</td>
<td>56.52±0.45</td>
<td>46.64±0.30</td>
<td>33.91±0.72</td>
<td>29.81±1.54</td>
<td>30.45±1.40</td>
</tr>
<tr>
<td>10</td>
<td>99.08±0.05</td>
<td>85.58±0.57</td>
<td>83.86±0.64</td>
<td>66.49±0.30</td>
<td>55.60±0.94</td>
<td>45.43±1.28</td>
<td>41.96±1.51</td>
<td>32.92±1.20</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>99.64±0.07</td>
<td>90.32±0.01</td>
<td>78.49±0.37</td>
<td>65.93±1.44</td>
<td>53.87±1.50</td>
<td>49.84±1.34</td>
<td>44.12±0.26</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
<td>99.87±0.04</td>
<td>84.53±0.33</td>
<td>71.39±1.24</td>
<td>66.54±0.33</td>
<td>59.32±1.24</td>
<td>54.87±0.50</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>87.61±0.51</td>
<td>75.10±1.34</td>
<td>69.89±0.55</td>
<td>67.03±0.37</td>
<td>60.17±0.89</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>90.65±0.37</td>
<td>80.17±1.16</td>
<td>75.13±0.71</td>
<td>69.70±0.51</td>
<td>66.08±1.08</td>
</tr>
</tbody>
</table>

mean±S.D, n=3
From the *in vitro* drug release study results, the optimum percentage drug release (90.65±0.37) at the end of 24h was observed with trial CL4 which contains 100mg of phospholipid.

Below 100mg of lipid concentration as in the case of trials CL1,CL2 and CL3, the maximum percentage drug release 99.08±0.05,99.64±0.07 and 99.87±0.04 were obtained at the end of 10h,12h and 16h respectively which was not desirable.

Beyond 100mg of lipid concentration, reduction in drug release was observed in the case of trials CL5-CL8. The maximum percentage drug release for CL5, CL6, CL7 and CL8 were found to be80.17±1.16, 75.13±0.71, 69.70±0.51 and 66.08±1.08 respectively at the end of 24h were obtained.

From the *in vitro* drug release data from CL1-CL8, it was observed that increase in the lipid concentration delays the drug release due to increased particle size and reduced surface area available for drug release.

**4. CONCLUSION**

The present work deals with development of liposomal drug delivery system for curcumin, an isolate from *curcuma longa*. Liposomes were prepared by using phospholipid as carrier. From the IR and DSC study results, it was identified that curcumin was compatible with other excipients used in the formulation. From all the formulations, CL4 was selected as best formulation due to its ideal particle size (245.82±3.26nm), high entrapment efficiency (90.63±0.29%) and desirable drug release (90.65±0.37% at 24h).

Further there is a need to improve the dissolution rate of the curcumin liposomes. One such ideal approach is to use an effective bioenhancer in the formulation. Hence in future, further work has to be carried out using a bioenhancer in curcumin formulation.

**REFERENCES**


