A Perspective View on Formulation and Optimization of Curcumin Loaded Phycocyanin Nanosponges

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ABSTRACT
Curcumin have a wide range of pharmacological activity and used in various disorders. Due to its poor bioavailability and fast metabolism peripherally, it is a major obstacle for researchers. Recent research on nanotechnology paved the way to overcome above concern and good results were obtained in formulation of curcumin using nanotechnology. The main objective of the study is to optimize, formulate & develop the curcumin loaded phycocyanin nanosponges and determine the variation in responses with respect to factors. The nanosponges were prepared using solvent evaporation technique and the final curcumin loaded Phycocyanin nanosponges (Cur-PC-NS) formulation is optimized and characterized. The formulation factors like drug polymer ratio, Aqueous/ Organic (A/O) phase ratio, concentrations of surfactant, Sonication & stirring time were considered as function excipient have shown different response values in aspects of Particle Size (PS), Zeta Potential (ZP), % of entrapment efficiency (EE%) & Loading capacity (LC%). In our findings F8 formulated nanosponges got 121.9nm particle size; -25.1mV zeta potential; 84.98% of Entrapment efficiency & 52.92% of loading capacity. Thus exhibited change in A/O phase ratio, surfactant concentration & Sonication time are 5:1, 0.5%, 1 hour respectively which have great impact on PS, EE% & LC%. We conclude that factor and its positive impact on response have to be taken in concern and utilized; we can develop better sustain release curcumin loaded phycocyanin nanosponges.

INTRODUCTION
Curcumin, a hydrophobic polyphenol derived from the rhizome of the herb Curcuma longa has multiple desirable characteristics for a neuroprotective drug, including anti-inflammatory, antioxidant, and anti-protein-aggregate activities. Because of its pluripotency, oral safety, long history of use, and inexpensive cost, curcumin has great potential for the prevention of multiple neurological conditions of use, and inexpensive cost, Curcumin has great potential for the prevention of multiple neurological conditions.

However Curcumin possess a strong intrinsic activity and thereby a therapeutic agent for various ailments, absorption, distribution, metabolism and excretion of curcumin have revealed poor absorption, rapid metabolism and clearance from the body and poor permeability of curcumin through the blood brain barrier is an obstacle to its future clinical applications.
Nanosponges are made up of microscopic particles with few nanometers wide cavities which can encapsulate number of substances. They are capable of carrying both lipophilic and hydrophilic substances which improve the solubility of poorly water soluble compounds (Vyasa and Khar, 2012; Shivani and Poladi, 2015). Target specific release of drug is done by nanosponges rather circulating throughout the body (Solunke et al., 2019).

Several reports have been published in last two decades describing the delivery of curcumin by various means such as micelles, liposomal formulations, nanoparticles and phospholipid complex showing enhanced bioavailability of curcumin and making the possible use of this compound for therapeutic prevention and reduction of risk at early stage of neurological disorders (Yallapu et al., 2013). Nanoparticles technology of curcumin is one of the frontier areas in medicine which will improve human health care. Interest in this area has been emerging worldwide over the last few years. A simplified and standardized approach is necessary to obtain curcumin nanoformulations. The process should not be extensive which would make the formulation costs minimal. Information of drug-excipient interactions, right excipient and also the right amount of the excipient could be a necessary prerequisite to the development of dosage forms that are stable and of fine quality (Yallapu et al., 2013; Selvamuthukumar and Velmurugan, 2014).

MATERIALS AND METHODS

Curcumin obtained from Sigma adrich, Phycocyanin from TCI chemicals Pvt. Ltd, Polyvinyl alcohol, double distilled water, Ethanol are analytical grade and gift sample obtained.

Preparation of Curcumin Loaded Phycocyanin Nanosponges (Cur-PC-NS)

Aqueous Phase: Phycocyanin Nanosponges (NS) was formulated using modified emulsion solvent diffusion method. First PVA (surfactant) is mixed in water and used for dissolving the weighed amount of phycocyanin which act as an aqueous phase.

Dispersed or Organic Phase: Defined amount of Curcumin is dissolved in sufficient quantity of ethanol and kept in sonicator for uniform dissolution, an organic phase.

Procedure

A total of 16 formulations Cur-PC-NS were formulated using modified emulsion solvent evaporation technique in trial and error method. The organic phase is poured into the aqueous phase in a uniform manner to ensure the proper mixing. The resultant solution is stirred for minimum 1 hour to 3 hours at 1200 RPM. Then the final product was filtered and dried, stored in air tight container. The percentage of 0.5% or 1%, drug polymer ratio, Sonication time, revolution per minute and duration of stirring time is changed (Srinivas and Sreeja, 2013; Velmurugan and Kasirajan, 2019).

Characterization

Particle size analysis was performed by dynamic light scattering with a Malvern Zetasizer 2000 HSA (Malvern Instruments, UK). Particle size analysis of Cur-PC-NS was performed using DLS (Malvern Zetasizer 2000, Malvern, UK) with a scattering angle of 90° at 25°C. The Cur-PC-NS sample was diluted in distilled water prior to measurement. Zeta potential is performed using DTS version 5.10 (Malvern Instruments, UK) & surface morphology (TEM-Transmission Electron Microscopy) is performed.

Determination of the Loading Capacity, Encapsulation Efficiency

Ultracentrifugation, 30 minutes at 4°C and 15,000 RPM, was used to estimate the entrapment efficiency (EE) and loading capacity (LC) of Cur-PC-NS. The developed HPLC method; acetonitrile (85%): 2 mM ammonium formate (15%): formic acid (0.1%) v/v/v with flow rate, 0.25 mL/min, was used to estimate and validate the free amount of curcumin in supernatant. The following equation, after triplicate measurements, is used to calculate EE and LC for developed Nanosponges:

\[
EE(\%) = \frac{(\text{total drug-free drug})}{\text{total drug}} \times 100
\]

\[
LC(\%) = \frac{(\text{total drug-free drug})}{\text{weight of NS}} \times 100
\]

Based upon drug polymer ratio, the LC obtained & EE obtained is shown the Table 2 . The observed data clearly proves Phycocyanin concentration in the entrapment process provides more negative charged, as seen by increased surface charge thus resulting stronger electrostatic attraction between Curcumin (positive charge) and Phycocyanin (negative charge). This polymer concentration leads to an increase in binding sites with high EE. This finding supports; greater the drug concentration, the lower the entrapment and higher LC (Ge et al., 2002).

RESULTS AND DISCUSSION

Particle Size and Zeta Potential Measurement by Dynamic Light Scattering

The nanosponges was prepared and characterized. Size of the particles in formulation was determined using DLS method and values were shown in Table 1. Our finding shows that after changing the quan-
tity ratio of factor like drug polymer ratio, aqueous organic phase, surfactant concentration, sonication time & stirring time and RPM (revolution per minute) in magnetic stirrer- the responses like particle size and zeta potential were different. In F8 & F14 formulation we used same factors but different responses similarly in F15 & F16 formulation we used same factors but different responses. We have got best result for F8 (Figure 1) of 121.9nm particle size (Figure 2) and -25.1mV zeta potential (Figure 3), which sufficient high enough for nanosponges to deliver drug. Even though the result were found to be satisfactory, retrials given as unexpected responses.

![Figure 1: Curcumin Loaded Phycocyanin Nanosponges (F8)](image1)

![Figure 2: Particle Size of Cur-PC-NS: F8 by Dynamic Light Scattering](image2)

![Figure 3: Zeta Potential of Cur-PC-NS: F8 by Dynamic Light Scattering](image3)

Loading Capacity, Entrapment Efficiency

The percentage entrapment efficiency (EE%) and drug loading capacity (LC%) was performed for 4 formulation & results shown in Table 2.

Effect of Factors on Responses

The ratio of aqueous formulation was changes from 10 fold to 3 folds ratio which produced a great impact on responses. It is not recommendable to increase more than 10ml of aqueous volume which may affect the nanodispersion and lower the Entrapment efficiency of drug (Selvamuthukumar and Velmurugan, 2014). The concentration of surfactant played important role. It is found that 0.5% of surfactant produced good result. Literature shows that increase in concentration not more than 1% is novel for having better stability and increase in EE% & LC%. But it was observed that in retrial we are unable get the same response on same factors (Budhian et al., 2007; Reddy et al., 2006; Song et al., 2008).

Surface Morphology: (TEM)

In order to provide information on the morphology of the Curcumin loaded phycocyanin nanosponges, Transmission Electron Microscopy was used to take photos. From the Transmission Electron Microscopy image (Figure 4) we can see that the obtained nanosponges are spherical, less aggregative, and relatively uniform in size.

List of Abbreviations

Table 1: Factors and Response

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug/Polymer Ratio</th>
<th>Aqueous/Organic (A/O) phase ratio</th>
<th>Surfactant %</th>
<th>Sonication Time</th>
<th>Particle Size (nm)</th>
<th>Zeta Potential (-mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1:2</td>
<td>10:1</td>
<td>1</td>
<td>10 minutes</td>
<td>1000-3000nm</td>
<td>-26.30mV</td>
</tr>
<tr>
<td>F2</td>
<td>1:1</td>
<td>2:1</td>
<td>0.5</td>
<td>10 minutes</td>
<td>1000-3000nm</td>
<td>-19.25mV</td>
</tr>
<tr>
<td>F3</td>
<td>1:1</td>
<td>5:1</td>
<td>1</td>
<td>30 minutes</td>
<td>217nm</td>
<td>-16.75mV</td>
</tr>
<tr>
<td>F4</td>
<td>1:1</td>
<td>2:1</td>
<td>0.5</td>
<td>30 minutes</td>
<td>386nm</td>
<td>-10.50mV</td>
</tr>
<tr>
<td>F5</td>
<td>1:2</td>
<td>3:1</td>
<td>0.5</td>
<td>30 minutes</td>
<td>189nm</td>
<td>-19.01mV</td>
</tr>
<tr>
<td>F6</td>
<td>1:3</td>
<td>4:1</td>
<td>1</td>
<td>30 minutes</td>
<td>386nm</td>
<td>-11.60mV</td>
</tr>
<tr>
<td>F7</td>
<td>1:4</td>
<td>5:1</td>
<td>1</td>
<td>30 minutes</td>
<td>282nm</td>
<td>-13.90mV</td>
</tr>
<tr>
<td>F8</td>
<td>1:1</td>
<td>5:1</td>
<td>0.5</td>
<td>1 hour</td>
<td>121.9nm</td>
<td>-25.1mV</td>
</tr>
<tr>
<td>F9</td>
<td>1:2</td>
<td>3:1</td>
<td>0.5</td>
<td>1 hour</td>
<td>176.4nm</td>
<td>-9.60mV</td>
</tr>
<tr>
<td>F10</td>
<td>1:2</td>
<td>5:1</td>
<td>0.5</td>
<td>1 hour</td>
<td>186.7nm</td>
<td>-15.01mv</td>
</tr>
<tr>
<td>F11</td>
<td>1:3</td>
<td>5:1</td>
<td>0.5</td>
<td>1 hour</td>
<td>198.2nm</td>
<td>-20.50mV</td>
</tr>
<tr>
<td>F12</td>
<td>1:4</td>
<td>3:1</td>
<td>0.5</td>
<td>1 hour</td>
<td>204.6nm</td>
<td>-13.60mV</td>
</tr>
<tr>
<td>F13</td>
<td>1:5</td>
<td>3:1</td>
<td>0.5</td>
<td>1 hour</td>
<td>220.7nm</td>
<td>-17.75mV</td>
</tr>
<tr>
<td>F14</td>
<td>1:1</td>
<td>3:1</td>
<td>0.5</td>
<td>1 hour</td>
<td>139.4nm</td>
<td>-19.60mV</td>
</tr>
<tr>
<td>F15</td>
<td>1:1</td>
<td>3:1</td>
<td>0.5</td>
<td>1 hour</td>
<td>154.2nm</td>
<td>-17.05mV</td>
</tr>
<tr>
<td>F16</td>
<td>1:1</td>
<td>3:1</td>
<td>0.5</td>
<td>1 hour</td>
<td>121.7nm</td>
<td>-25.1mV</td>
</tr>
</tbody>
</table>

Table 2: Loading Capacity and Entrapment Efficiency

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>EE%</th>
<th>LC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>F8</td>
<td>84.98%</td>
<td>52.92%</td>
</tr>
<tr>
<td>F14</td>
<td>87.25%</td>
<td>35.20%</td>
</tr>
<tr>
<td>F15</td>
<td>89.20%</td>
<td>36.00%</td>
</tr>
<tr>
<td>F16</td>
<td>94.98%</td>
<td>47.80%</td>
</tr>
</tbody>
</table>

Performance Liquid Chromatography, mL/min - Milliliter/minute, nm – Nanometer, mV – millivolts, mM – Millimole, v/v – Volume per volume

CONCLUSION

Our study concludes that the curcumin can be loaded in phycocyanin nanosponges which have good characterization results. To overcome the poor knowledge on drug-exciient/Variables/factors interaction, the negative or undesirable results and its reason our research have to be considered and used for the future study.

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Conflicts of Interest

The authors declare that they have no conflict interest.

Consent of Ethics

No experiment is performed on animals or human volunteers for above formulation work.

REFERENCES


