Preparation and evaluation of Maltodextrin based Proniosomes containing Capecitabine

Srikanth¹, Y. Anand Kumar *¹ and C. Mallikarjuna Setty²

¹ Department of Pharmaceutics, V.L. College of Pharmacy, Raichur, India
² Oxford College of Pharmacy, Bengaluru, India

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ABSTRACT: Maltodextrin based capecitabine proniosomes were fabricated by conventional slurry method with different surfactant: cholesterol molar ratio keeping constant capecitabine and dicetyl phosphate concentration. The fabricated proniosomes were evaluated for FTIR and flow properties. Further proniosomes were hydrated to get desired niosomes and the obtained niosomes were subjected for various evaluation parameters such as entrapment efficiency, in vitro drug release and stability studies as per ICH guidelines. The entrapment efficiency followed the trend, span 80 (C₁₀) > span 20 (C₁₂) as increasing the alkyl chain lengths are leading to higher entrapment efficiency. The in vitro data suggest biphasic drug release, initial burst followed steady state. In all the case, best-fit model was found to be matrix with Peppas exponential value was greater than 0.5 indicating drug released by non Fickian (anomalous) mechanism. The result of investigation demonstrated that proniosomes offers an alternate drug carrier with targeted specificity.

INTRODUCTION

Research in the field of novel drug delivery system, which continues to progress rapidly, aims at the development of drug delivery systems (DDS) with optimum therapeutic benefits including safe and effective management of disease [1]. The concept of drug delivery to a specific site for the treatment of localized disease in the body, thereby decreasing drug adverse effects and improving its therapeutic index, is often considered a challenge [2]. The idea of a drug carrier with targeted specificity has always fascinated scientists for decades and in the last decade, limited success have been achieved in this regard. One such approach involves the use of vesicular drug carrier that can provide site specificity combined with optimal drug release profile [3]. Amongst various carriers utilized for target-oriented drug delivery, vesicular drug delivery systems in the form of liposome and niosomes have been most extensively investigated. Liposomal formulations have the limitation of poor stability and low drug entrapment efficiency while niosomes exhibit physical instability, aggregation, fusion, and leakage of entrapped drug, thus limiting the shelf-life of the dispersion [4,5]. Proniosomes are free flowing dry product which could be hydrated immediately before use, avoid many of the problems associated with aqueous niosome dispersions and problems of physical stability viz., aggregation, fusion, leaking [6]. Proniosomes are dry formulations of surfactant coated water-soluble carrier particles and can be hydrated easily by simple agitation in hot aqueous media to form multilamellar niosome dispersions suitable for administration by oral or other routes [7,8]. Capecitabine is an orally administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers [9]. Chemically it is a prodrug of 5'-deoxy-5-fluorouridine (5'-DFUR) figure 1, which is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue [10,11].
The objective of the present research work is to develop a vesicular drug delivery system for capecitabine in the form of proniosomes using maltodextrin as carrier and different grades of spans as surfactant, which will have advantages of controlled drug release, increased drug stability and high drug load.

**Figure 1: Chemical structure of capecitabine.**

**MATERIALS AND METHODS**

**Materials:** Capecitabine gift sample was obtained from Shilpa antibiotic Pvt Ltd, Raichur. Maltodextrin was procured from Himedia, Hosur, Cholesterol, Span 80, Span 20 and DCP (Dicetyl phosphate) were purchased from Loba Chem Pvt Ltd, Mumbai. All the other ingredients and reagents used were of analytical grade.

**Table 1: Different formulae of maltodextrin based capecitabine proniosomes.**

<table>
<thead>
<tr>
<th>Batches</th>
<th>Surfactant</th>
<th>Drug (mg)</th>
<th>Surfactant : Cholesterol Molar ratio</th>
<th>Surfactant (mg)</th>
<th>Cholesterol (mg)</th>
<th>Maltodextrin (2 G)</th>
<th>DCP (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Span 80</td>
<td>50</td>
<td>1:1</td>
<td>430.62</td>
<td>386</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>B2</td>
<td>Span 80</td>
<td>50</td>
<td>1:0.75</td>
<td>430.62</td>
<td>293</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>B3</td>
<td>Span 80</td>
<td>50</td>
<td>1:0.50</td>
<td>430.62</td>
<td>193</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>B4</td>
<td>Span 80</td>
<td>50</td>
<td>1:1</td>
<td>346.45</td>
<td>386</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>B5</td>
<td>Span 20</td>
<td>50</td>
<td>1:0.75</td>
<td>346.45</td>
<td>289</td>
<td>2</td>
<td>7.5</td>
</tr>
<tr>
<td>B6</td>
<td>Span 20</td>
<td>50</td>
<td>1:0.50</td>
<td>346.45</td>
<td>193</td>
<td>2</td>
<td>7.5</td>
</tr>
</tbody>
</table>

**EVALUATION**

**FTIR studies:** The FTIR spectra for capecitabine, maltodextrin, span80, span20 and selected proniosome formulations were recorded. The samples were prepared in KBr disks prepared with a hydrostatic press at a force of 5.2Tcm-2 for 3min. The scanning range was 450-4000cm-1 and the resolution was 1cm-1.

**Angle of repose [14]:** The angle of repose of dry proniosomes powder and maltodextrin powder was measured by a cut funnel method. The maltodextrin powder and proniosomes powder was poured into a funnel which was fixed at a position so that the 13mm outlet orifice of the funnel is 5cm above a level black surface. The powder flows down from the funnel to form a heap on the surface and the angle of repose was then calculated by measuring the height of the heap and the diameter of its base. Angle of repose was calculated by using following formula.

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

Where, \( \theta \) - Angle of repose; \( h \) - Height of the heap; \( r \) - Radius of the heap

**Preparation of proniosomes:** Six maltodextrin based capecitabine pronosome formulations were fabricated using surfactants viz., span80 and span20: cholesterol at 1:1; 1:0.75 and 1:0.50 molar ratio by conventional slurry method. The different formulae were given in table1.

**Slurry method [12, 13]:** A 250µmol stock solution of Span 80, Span 20, cholesterol and dicetyl phosphate was prepared in chloroform: methanol (2:1). The accurately measured volumes of span 80, cholesterol, dicetyl phosphate stock solutions and capecitabine (50mg) dissolved in chloroform: methanol (2:1) solutions were added into a 250ml round bottom flask containing previously 2g of maltodextrin powder as carrier. Additional chloroform: methanol (2:1) solution added to form slurry. Further the flask was attached to a rotary flash evaporator rotated at 60 to 70 rpm. The solvent is allowed to evaporate at temperature of 45±2ºC in a reduced pressure of 600mm/Hg until the mass in the flask had become a dry, free flowing product. The obtained proniosome powder was further dried overnight in a desiccator under vacuum at room temperature. Similarly, another batch of proniosome was prepared using Span 20 by adapting the same procedure as described above. The obtained dry proniosome powders were stored in air tight amber colored vials kept in a refrigerator for further evaluation.

**Preparation of niosomes:** Niosomes were prepared for all the fabricated proniosomes by simple hydration method. In this method accurately weighed proniosome formulations were filled in series of vials to this add measured volume of phosphate buffer pH 7.4, the components are mixed for 2min on vortex mixer followed by sonication for 30sec to get desired niosomes. The prepared niosomes were stored in air tight container for further evaluation.

**Drug content:** Niosomes equivalent to 50 mg of capecitabine were extracted with 25ml of distilled water in a 100ml volumetric flask further, it was made up to 100ml and keep undisturbed for 30 minutes to achieve complete extraction. The extract was filtered and diluted serially with phosphate buffer pH 7.4 and the absorbance was measured at 303 nm thus drug content was calculated from the calibration curve and Average of three readings were taken and computed.

**Entrapment efficiency:** Niosome entrapped capecitabine was estimated by dialysis method. The calculated amount of prepared niosomes was placed in the dialysis bag (presoaked for 24 hrs). Free capecitabine was dialyzed for 30 minutes each time in 100 ml of phosphate buffer pH 7.4.
The dialysis of free capecitabine always completed after 12-15 changes, when no capecitabine was detectable in the recipient solution. The dialyzed capecitabine was determined by finding out the concentration of bulk of solution by UV spectrophotometer at 303 nm. The samples from the bulk of solution diluted appropriately before going for absorbance measurement. The free capecitabine in the bulk of solution gives us the total amount of unentrapped drug. The percentage entrapment efficiency is calculated by using following formula,

\[
\text{% Entrapment efficiency} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug}} \times 100
\]

Particle size distribution and average particle size determination: Particle size analysis was carried out using an optical microscope (compound microscope) with a calibrated eyepiece micrometer.

Calibration of eye piece micrometer: A standard stage micrometer was used for calibration. Each division value on stage is 10µ. The eye piece micrometer consists of 100 divisions. Calibration was undertaken to find out the measure of each division using the standard stage micrometer. After calibration, the eye piece micrometer was used for particle size determination. A drop of niosomal preparation was mounted on a slide and observed under the microscope. About 200 niosomes were measured individually with the help of eye piece micrometer, average was used to plot size distribution curve and calculate average mean diameter.

Microphotography: The vesicle formation by the hydration process was confirmed by mounting niosome preparation on a slide and observed under the optical microscopy at 200x resolution. The microphotomicrographs of the niosomes were recorded by using a digital SLR camera.

In vitro release study: All the niosome formulations were subjected for in vitro drug release study by using dialysis bag method. 10 mg equivalent of capecitabine niosome preparation was taken in dialysis bag and the bag was placed in a beaker containing 75 ml of phosphate buffer pH 7.4. The beaker was placed over magnetic stirrer having stirring speed of 100 rpm and the temperature was maintained at 37±1°C. At a predetermined interval of time 5ml samples were withdrawn and same were replaced with phosphate buffer pH 7.4 to maintain the sink condition throughout the release period. The withdrawn samples were appropriately diluted with phosphate buffer pH 7.4 and analyzed for drug content using UV spectrophotometer at 303nm keeping phosphate buffer pH 7.4 as blank. The diffusion studies were carried out in triplicate and the data were interpreted, model fitted by using dissolution software PCP-DISSO V.3.

Stability Study: Physical stability study was carried out to investigate the degradation of drug from proniosome during storage as per ICH guideline. Best two of the optimized capecitabine proniosome formulations composed of spans and cholesterol sealed in glass vials and stored in refrigerated temperature (2-8°C) and room temperature for a period of 3 months. Samples from each batch were withdrawn after the definite time intervals and converted into niosome formulations and determine the entrapment efficiency, drug content and in vitro drug release and interpret the data.

RESULTS AND DISCUSSION

FTIR study: The drug excipient compatibility studies were done by FTIR and comparative spectra were shown in figure 2. The FTIR of capecitabine shows a characteristic band of -NH stretching at 3516.49 cm⁻¹, -CH stretching at 2959.47 cm⁻¹ and C=O stretching at 1710.90 cm⁻¹.

The characteristic capecitabine -NH stretching band was observed in selected proniosomes in the range of 3426.75 cm⁻¹ to 3423.33 cm⁻¹; -CH stretching 2999.38 cm⁻¹ to 2957.72 cm⁻¹ and C=O stretching 1742.23 cm⁻¹ to 1737.63 cm⁻¹.
Drug content: The percentage drug content was found to be in the range of 99.10± 0.32, 99.70± 0.11, 99.20 ± 0.42, 99.00 ± 0.61, 99.60 ± 0.41 and 99.30 ± 0.11 for B1 to B6 formulations. The low standard deviation (SD) and low coefficient of variation (CV) i.e.<2 indicates drug distribution was uniform in all the niosome formulations.

Morphology Study: Shape and surface characteristic of maltodextrin based proniosome formulations were converted into niosomal suspensions were studied under optical microscope at 200x magnifications to observe the formation of vesicles and are shown in figure 4 and figure 5.

Entrapment efficiency study: The percentage entrapment efficiency was found to be in the range of 76.91 ± 0.54 to 92.06 ± 0.18 for S1 to S6. The data was given in table 2 and profile in figure 6.

Stability study: Stability studies of all prepared niosomes were performed by storing 4°C, 25°C and 37°C for a period of 3 months. The data was given in table 4.
DISCUSSION

The FTIR results suggest the characteristic absorption bands of capecitabine were shifting little toward lower/higher wavelength indicating minor or no interaction. The angle of repose of dry proniosome powder is smaller than that of pure maltodextrin and the values obtained were within the standard limit of flowability. The particles were found to be uniform in size and shape and the size distribution was in the range of 3 to 8 µm and the particle size of the niosomes was found to be in the range of 4.45µ, 4.81µ, 4.36µ, 4.79µ, 4.93µ and 4.69µ for B1 to B 6 formulations respectively, microphotographs of niosome formulations reveals that the niosomes were spherical in their shape. All span types have the same head group and different alkyl chain. Increasing the alkyl chain length is leading to higher entrapment efficiency. The entrapment efficiency followed the trend span 80 (C18) > span 20 (C12). In addition, span 80 has the lowest transition temperature (Tc- -12°C) when compared to span 20 i.e. 16°C. In both the cases the as the concentration of cholesterol increases the entrapment efficiency decreases.

In niosome formulations, the drug release was found to be 20.96%, 18.15% and 18.15% for span 80 formulations similarly 24.03%, 20.76 and 13.61 % for span 20 formulations at the end of 1.5 hrs. This initial burst release was mainly due to improper formation or any adherence of drug particles around the niosomes and release of adsorbed drug from the lipophilic region of niosomes.

Fast drug release in the initial hours may help to achieve the optimal loading dose. Further, the drug release follows a biphasic drug release, up to 6 hrs the drug release follows first order and at the start of 12hrs the release was found to be steady because stable niosomes retains and the release was extended up to 36hrs with sustained action. Increasing cholesterol concentration markedly reduced the efflux of the drug and fills the pores in vesicular bilayer and abolishes the gel-liquid phase transition of niosome systems resulting in less leakage of drug from niosomes. This confirms that cholesterol in the formulation acts as a membrane stabilizing agent that helps to sustain drug release.

Table 3: Model fitting data for B1 to B6 formulations

<table>
<thead>
<tr>
<th>Model fitting values</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
<th>B6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td>0.7380</td>
<td>0.7641</td>
<td>0.7252</td>
<td>0.7690</td>
<td>0.7785</td>
<td>0.8400</td>
</tr>
<tr>
<td>1st order</td>
<td>0.9449</td>
<td>0.9362</td>
<td>0.8997</td>
<td>0.9897</td>
<td>0.9218</td>
<td>0.9728</td>
</tr>
<tr>
<td>Matrix</td>
<td>0.9772</td>
<td>0.9757</td>
<td>0.9672</td>
<td>0.9883</td>
<td>0.9845</td>
<td>0.9887</td>
</tr>
<tr>
<td>Peppas</td>
<td>0.9627</td>
<td>0.9618</td>
<td>0.9581</td>
<td>0.9660</td>
<td>0.9668</td>
<td>0.9706</td>
</tr>
<tr>
<td>Hix.Crow. n</td>
<td>0.8986</td>
<td>0.8953</td>
<td>0.8565</td>
<td>0.9535</td>
<td>0.9330</td>
<td>0.9448</td>
</tr>
<tr>
<td>k</td>
<td>0.6237</td>
<td>0.6754</td>
<td>0.6662</td>
<td>0.6861</td>
<td>0.6320</td>
<td>0.6766</td>
</tr>
<tr>
<td>Best fit</td>
<td>Matrix</td>
<td>Matrix</td>
<td>Matrix</td>
<td>Matrix</td>
<td>Matrix</td>
<td>Matrix</td>
</tr>
</tbody>
</table>

Table 4: Stability study data for B1to B6 formulations

<table>
<thead>
<tr>
<th>Batches</th>
<th>% Drug content±SD</th>
<th>% Entrapment efficiency ±* SD</th>
<th>Cumulative percent drug release ± *SD (after 36hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B3</td>
<td>99.10 ± 0.32</td>
<td>82.69 ± 0.21</td>
<td>77.37± 0.39</td>
</tr>
<tr>
<td>B6</td>
<td>99.20 ± 0.21</td>
<td>91.00 ± 0.32</td>
<td>84.28± 0.22</td>
</tr>
</tbody>
</table>
The in vitro drug release data was model fitted with various models and the result suggest best fit model was found to be matrix with Peppas exponential ‘n’ value was greater than 0.5 suggesting the drug was released by non Fickian (anomalous) mechanism i.e., the drug released by erosion followed by diffusion controlled. The residual drug content was determined at the end of third month. It was observed that the drug leakage from the vesicles was least at 4°C followed by 25°C and 37°C. This may be attributed to phase transition of surfactant and lipid causing vesicles leakage at higher temperature during storage. Hence it is concluded from the obtained data that the optimum storage condition for niosomes was found to be 4°C.

CONCLUSION

Maltodextrin based capecitabine proniosomes can be conveniently prepared by conventional slurry method with negligible loss of drug and further it is convenient to convert into desired niosome by simple hydration process. The evaluation studies conclude that niosomes are superior in their convenience of storage, transport and dosing as compare to niosomes prepared by conventional method. The result of investigation demonstrated that proniosome offer an alternate colloidal carrier approach in achieving drug targeting.

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