FORMULATION AND EVALUATION OF LIPOSOMES CONTAINING ANTI-CANCER DRUG DECITABINE
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ABSTRACT
Liposomes, which are biodegradable and essentially non-toxic vehicles, can encapsulate both hydrophilic and hydrophobic materials, and are utilized as drug carriers in drug delivery systems. In addition, liposomes can be used to carry radioactive compounds as radiotracers can be linked to multiple locations in liposomes. The aim of this study was to Formulation and In-vitro evaluation of liposomal drug delivery system of Decitabine is an anticancer medication is indicated for treatment of patients with myelodysplastic syndrome (MDS). Decitabine Liposomes are prepared by the thin film hydration method using the soya lecithin as the phospholipid. The prepared liposomes were characterized by scanning electron microscopic method respectively. The In-vitro release studies were performed and the drug release kinetics was evaluated using linear regression method. The objective of the present study was to develop liposome containing Decitabine and the prepared liposomes were evaluated for size, shape, drug entrapment efficiency, In-vitro drug release and stability. The intestinal absorption and antitumor capacity of Decitabine was significantly enhanced by using liposomes.

Key Words: Liposomes, (MLV) multi lamellar vesicles, Thin film hydration method, Decitabine, Lecithin, Cholesterol, Tween 80.

INTRODUCTION
Rational research in drug delivery began in 1950’s with the advent of polyclonal antitumor antibodies developed for tumour targeting of cytotoxic drugs to experimental tumors. Liposomes were discovered in the early 1960’s by British haematologist Dr Alec D Bangham (published 1965) and subsequently became the most extensively explored drug delivery system [1-8]. At first they were used to study in vivo simulated bio membrane behaviour. Subsequent to that liposome has become an essential therapeutic tool most notably in drug delivery and targeting. Not surprisingly, liposomes have covered predominantly medical, non-medical areas like bioreactors, catalysts, cosmetics and ecology. However, their predominance in drug delivery and targeting has enabled them to be used as therapeutic tool in fields like tumour targeting, gene and antisense therapy, genetic vaccination, immune modulation, lung therapeutics, fungal infections, and skin care and topical cosmetic products . The name liposome is derived from two Greek words: ‘Lipos’ meaning fat and ‘Soma’ meaning body [9-12]. Liposomes are spherical microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer
compartments with a diameter ranging from 25 nm to 10000 nm. They are commonly composed of one or more amphiphilic phospholipids bilayer membranes (and thus also called as phospholipid vesicles) that can entrap both hydrophilic and hydrophobic drugs [13-18]. A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drug or genetic material into a cell. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chain like egg phosphatidyl ethanolamine or of pure components like DOPE (dioleoyl phosphatidyl ethanolamine). Decitabine is indicated for treatment of patients with myelodysplastic syndrome (MDS). Half-life is 30 mins decitabine is slightly soluble in ethanol/water, methanol/water sparingly soluble in water soluble in dimethylsulfoxide. Decitabine liposome’s were prepared using soya lecithin, cholesterol, Tween80, and chloroform as solvent by thin film hydration method using rotary evaporator. The prepared Liposomes were evaluated by drug entrapment study, particle size analysis. In vitro drug release study, mechanism of release kinetics using Higuchi’s plot and korsemeyer Peppas plot and stability studies [19-20].

MATERIALS AND METHODS
Decitabine was obtained from Aurobindo laboratories Ltd, Soyabean lecithin was purchased from Bridge pharmaceuticals Pvt. Ltd, Hyderabad. The materials used in the present investigation were either AR/LR grade or the best possible pharma grade.

Preparation
The preparation of liposomes with Soybean lecithin was prepared by dried thin film hydration technique using a rotary evaporator (Aditya scientific). Drug, Soya lecithin, cholesterol, tween 80 and were dissolved in 10 mL chloroform in 250mL round bottom (RB) flask. The chloroform was evaporated under vacuum using rotary flash evaporator 65-70°rom, which allows soya lecithin to form a thin dry film on the walls of the flask. This system was maintained at vacuum and 40°C for an additional 10min, after complete removal of organic solvent as indicated by visual observations. Vesicles were prepared by hydrating the lipid film in the presence of 10mL phosphate buffer pH 7.4. Liposomes formed were sonicated for 30 min. to reduce the size of the vesicles. The composition and ratios of lecithin, cholesterol and Tween 80 for different types of Liposomes were mentioned in Table No. 1

Table 1: Qualitative and Quantitative Lipid Compositions of Different Formulations

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F-1</th>
<th>F-2</th>
<th>F-3</th>
<th>F-4</th>
<th>F-5</th>
<th>F-6</th>
</tr>
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<tbody>
<tr>
<td>Drug(mg/ml)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Soya lecithin (mg)</td>
<td>240</td>
<td>270</td>
<td>210</td>
<td>180</td>
<td>210</td>
<td>180</td>
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<tr>
<td>Cholesterol(ml)</td>
<td>60</td>
<td>30</td>
<td>90</td>
<td>120</td>
<td>90</td>
<td>120</td>
</tr>
<tr>
<td>Chloroform(ml)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Tween 80(ml)</td>
<td>---</td>
<td>0.5</td>
<td>---</td>
<td>---</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>PBS 7.4(ml)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Hydration time</td>
<td>20</td>
<td>35</td>
<td>30</td>
<td>20</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Fig. 1: Representation of % Entrapment Efficiency Of Different Formulations

The percentage entrapment was maximum F2 is 82.96% and minimum for F4 is 78.39%. The data suggests that concentrations with respect to the formulation represent the critical value up to which the entrapment increased and beyond that its start decreasing.

Fig 2: SEM Photography of Liposomal Solution for F2 Formulation

The shape and morphology of the liposome droplet was determined by SEM show the round shape, smooth surface and nano size range of vesicle. Demonstrating Multi lamellar vesicles structure under electron microscopic study confirming the vesicle characteristics.
Table 2: In-Vitro Cumulative % Drug Release Profile of Decitabine Liposomal Formulations

<table>
<thead>
<tr>
<th>Time(Hr)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>1</td>
<td>8.29</td>
<td>5.68</td>
<td>10.65</td>
<td>12.74</td>
<td>13.11</td>
<td>15.13</td>
</tr>
<tr>
<td>2</td>
<td>15.36</td>
<td>12.21</td>
<td>20.21</td>
<td>25.31</td>
<td>26.05</td>
<td>28.06</td>
</tr>
<tr>
<td>4</td>
<td>33.53</td>
<td>28.77</td>
<td>39.85</td>
<td>40.5</td>
<td>42.92</td>
<td>45.01</td>
</tr>
<tr>
<td>6</td>
<td>58.21</td>
<td>51.65</td>
<td>63.56</td>
<td>68.9</td>
<td>67.53</td>
<td>68.62</td>
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<tr>
<td>10</td>
<td>78.85</td>
<td>74.25</td>
<td>81.56</td>
<td>83.78</td>
<td>84.86</td>
<td>85.07</td>
</tr>
<tr>
<td>12</td>
<td>95.85</td>
<td>89.46</td>
<td>92.59</td>
<td>94.84</td>
<td>95.25</td>
<td>93.67</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
<td>96.31</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99.86</td>
</tr>
</tbody>
</table>

Fig 3: In Vitro Drug Release Study of F1 to F6

The in vitro dissolution profile prepared formulations was determined by membrane diffusion method. The dissolution was carried out for a period of 24 hrs in 7.4 pH phosphate buffer. The cumulative percent release of F1 to F6 formulations at various time intervals was calculated and tabulated in table. The cumulative percent drug release in all formulations was plotted against time in Figure. The Maximum percent of drug release was found in F2 formulation which contains maximum drug entrapment.
RELEASE KINETICS

**Fig. 4:** Zero Order Plot for Optimised Formulation

\[ y = 7.627x - 0.7035 \]
\[ R^2 = 0.9925 \]

**Fig. 5:** First Order Plot for Optimised Formulation

\[ y = -0.0769x + 2.0826 \]
\[ R^2 = 0.9415 \]

**Fig 6:** Higuchi Plot for Optimised Formulation

\[ y = 27.409x - 15.39 \]
\[ R^2 = 0.9158 \]
STABILITY STUDIES: The liposomal formulation was tested for a period of 8 weeks at different temperatures of 25°C and 40°C with 60% RH and 75%RH for their drug dissolution.

**Table 3**: Stability Dissolution Results of Optimized Formulation-F2

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Parameters</th>
<th>Initial drug release after 24 hrs</th>
<th>After 1st Month</th>
<th>After 2nd Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2</td>
<td>25°C/60% RH % Release</td>
<td>96.31</td>
<td>96.3</td>
<td>96.29</td>
</tr>
<tr>
<td>F2</td>
<td>30°C/75% RH % Release</td>
<td>96.31</td>
<td>96.29</td>
<td>96.28</td>
</tr>
<tr>
<td>F2</td>
<td>40°C/75% RH % Release</td>
<td>96.31</td>
<td>96.28</td>
<td>96.28</td>
</tr>
</tbody>
</table>
CONCLUSION

The Decitabine loaded liposome formulation had good ability to encapsulate drug and elicited favorable physicochemical characteristics. The intestinal absorption and antitumor capacity of Decitabine was significantly enhanced by using liposomes. These results suggest that liposomes could be a promising perioral carrier for Decitabine. From the executed experimental results, it could be concluded that the lipids like Soya lecithin, Cholesterol and Tween80 were suitable carrier for the preparation of Decitabine Liposomes. Though the preliminary data based on in-vitro dissolution profile, release kinetics and stability studies proved that the suitability of such formulations, still a thorough experiment will be required based on the animal studies. Thereafter we can find the actual mode of action of this kind of dosage form. Therefore, a future work will be carried out as follows,

- Long term stability studies
- In-vitro Cytotoxicity studies
- In-vivo Pharmacological work on animals.
- In-vivo pharmacokinetic studies on animals.

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