Preparation and evaluation of a novel liposomal cyclosporin A using the supercritical fluid method

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ABSTRACT SUMMARY
A novel method to prepare liposomal cyclosporin A (CsA) was developed using the supercritical fluid of carbon dioxide (SCF-CO2) method.

Comparing to the conventional liposomes prepared by modified conventional Bangham method, SCF-CO2 liposomes were relatively smaller, more homogeneous and physically and chemically more stable. This simple and innovative method might be scaled-up easily. Therefore, the newly developed SCF-CO2 method could be the better alternative to the conventional method for large-scale production of liposomes.

INTRODUCTION
Liposomes have been investigated for the delivery of CsA in order to overcome the problems associated with conventional delivery systems and to improve the efficacy of CsA. Presently available methods to prepare liposomes are mainly suitable for laboratory-scale and less for industrial approach. Some modified laboratory-scale methods e.g. ethanol injection, cross-flow filtration, dialysis, spray-drying etc, have been studied and attempted for large-scale preparation, but still they have some issues, mainly residual solvents in the final product, that make these methods less applicable.

Castor (1994) first discovered liposome preparation method using green and non-toxic, SCF-CO2 and since then there are number of studies supported the possible application of SCF-CO2 method of liposome preparation for large-scale. The present method utilized in this study is a novel and relatively simple compared to the other existing SCF-CO2 methods. This method is based on the supercritical anti-solvent technique, in which phospholipid-drug-cholesterol mixture is coated on the surface of the career particle (anhydrous lactose) forming a thin film, which on hydration gives MLVs. The objective of this study was to compare the physiochemical properties and stabilities of liposomes prepared by novel SCF-CO2 and conventional modified Bangham methods.

EXPERIMENTAL METHODS
Liposomes were prepared by both conventional and SCF-CO2 methods. For conventional method, 200 mg of phosphatidylcholine (PC- Lipoid S100 or Lipoid E80), 100 mg of cholesterol and 50 mg of CsA were dissolved in 2.5 ml of ethanol and solution was transferred to round-bottom flask containing 900 mg of anhydrous lactose, which was connected to the rotary evaporator. The organic solvent was then slowly removed under reduced pressure and temperature (45°C), thus forming a thin film on the surface of the flask. The resulting thin film was then hydrated with 10 ml Milli-Q water to form MLVs.

For SCF-CO2 method, same composition of PC, cholesterol and CsA was dissolved in 2.5 ml of ethanol and sealed in the reaction vessel with 900 mg of anhydrous lactose. The SCF-CO2 was pumped to the vessel, where optimum pressure and temperature of the process was 45°C and 10 MPa, respectively. After complete removal of the organic solvent, the vessel was slowly depressurized to atmospheric pressure, and CsA-lipid-cholesterol mixture coated on the surface of the lactose particles forming a thin film. After hydrating thin film at 50°C, MLVs were obtained.

Liposomes prepared by both methods were further characterized. The CsA content in liposomes (yield) was measured by RP-HPLC. Entrapment efficiency (EE) and drug loadings (DL) properties of liposomes were analyzed by ultracentrifugation method. The particle size, zeta potential and polydispersity index (PDI) of liposomes were measured by particle size analyzer at room temperature. Transmission electron microscopy was also performed to study the lamellarity and shape of SCF-CO2 liposomes.

For chemical and physical stability studies, liposomes were kept refrigerated at 4°C and, at predetermined times over a period of 3 months, the parameters of size, PDI, yield, EE% and DL% of liposomal suspensions were analyzed. The effect of lyophilization on the liposomes was evaluated using cryoprotectant and without cryoprotectant.

Figure 1. Schematic representation of the experimental apparatus for liposome preparation by SCF-CO2 method.
RESULTS AND DISCUSSION

CsA encapsulated liposomes were successfully prepared by both methods. The optimum condition of pressure and temperature was found to be 10 MPa and 45°C for liposome preparation by SCF-CO₂ method. The results of the particle size measurement by DLS indicated that liposomes prepared with SCF-CO₂ were relatively much smaller (p<0.02) and more homogenous in size (Table 1). CsA is highly lipophilic compound and hence, showed approximately 90% of EE, regardless of the preparation method. TEM images confirmed that SCF-CO₂ liposomes were multilamellar and spherical in shape.

Table 1. Characteristics of liposomes.

<table>
<thead>
<tr>
<th>Liposome</th>
<th>Mean Diameter (nm)</th>
<th>Polydispersity Index (PDI)</th>
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<tr>
<td>Non-filtered</td>
<td></td>
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<tr>
<td>SCF-S100</td>
<td>1137.96±131.39</td>
<td>0.34±0.02</td>
</tr>
<tr>
<td>SCF-E80</td>
<td>747.04±82.30</td>
<td>0.30±0.03</td>
</tr>
<tr>
<td>Film-S100</td>
<td>2155.90±373.55</td>
<td>0.61±0.09</td>
</tr>
<tr>
<td>Film-E80</td>
<td>1543.01±66.31</td>
<td>0.51±0.10</td>
</tr>
<tr>
<td>Filtered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCF-S100</td>
<td>188.93±17.00</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>SCF-E80</td>
<td>166.58±9.60</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Film-S100</td>
<td>244.30±44.15</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>Film-E80</td>
<td>198.87±26.24</td>
<td>0.20±0.03</td>
</tr>
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Liposomes were filtered by 1.0 µm pore-sized PTFE filter in order to avoid interferences by large aggregates or dust particles. The initial size of SCF-CO₂ liposomes prepared by Lipoid S100 and Lipoid E80 was 198.75±10.57 nm and 164.10±8.47 nm, respectively. After 14 weeks of storage at 4°C, the particle size changed to 195.77±9.02 nm for SCF-S100 and 169.25±4.05 nm for SCF-E80, respectively (Fig. 2). It was interesting to note that SCF-CO₂ liposomes did not show significant changes in particle size (p>0.05) or PDI (p>0.05) during 14 weeks, indicating that there was no aggregation. On the contrary, conventional liposomes showed the irregular behavior of particles sizes and PDI during 13 weeks of storage at 4°C.

The initial percentages of yield, EE, and DL were 90.98±2.94, 92.20±1.36 and 20.99±0.84, respectively, for SCF-S100 liposomes. Even after 14 weeks of storage at 4°C, there were no significant changes (p>0.05) in any of the parameters tested. On the contrary, Film-S100 liposomes had significant reductions in yield (%) from 90.72±2.83 to 75.04±8.80, EE (%) from 90.24±1.37 to 84.59±5.13 and DL (%) from 20.47±0.94 to 15.94±2.80 after 14 weeks of storage at 4°C (Fig. 2). Statistically, there were significant differences (p<0.05) between the two formulations, suggesting that SCF-CO₂ liposomes prepared by Lipoid S100 were much more stable than the liposomes formed by the conventional method.

CONCLUSION

SCF-CO₂ method provides a simpler and more efficient way to prepare liposomes with a characteristically smaller size and better morphology together with improved entrapment efficiency and drug loading. It also shows better stabilities. These results indicate that liposomes prepared by the SCF-CO₂ method were relatively more stable compared with those prepared by the conventional method, which was in accordance with the results by Otake et al.² and Kadimi et al.³ The stability of liposomes prepared with SCF-CO₂ might be explained by the static repulsion of the carbonic acids incorporated into the bilayer membrane.

REFERENCES


ACKNOWLEDGMENTS

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