Liposome Formation: Hydrogenated Egg Lecithin

Summary of Operating Conditions

*Table 1. Summary of Operating Conditions*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Incident Power</td>
<td>150 W</td>
</tr>
<tr>
<td>Duty Factor</td>
<td>50%</td>
</tr>
<tr>
<td>Cycles per Burst</td>
<td>1000</td>
</tr>
<tr>
<td>Duration</td>
<td>3600 seconds</td>
</tr>
<tr>
<td>Water Bath Temperature</td>
<td>3°C</td>
</tr>
<tr>
<td>Power Mode</td>
<td>frequency sweeping</td>
</tr>
<tr>
<td>Degassing Mode</td>
<td>continuous</td>
</tr>
<tr>
<td>Volume</td>
<td>2 mL</td>
</tr>
</tbody>
</table>

Supplies

*Table 2. Equipment List*

<table>
<thead>
<tr>
<th>Part Name</th>
<th>Description</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Vessel</td>
<td>Tube &amp; Cap 12x24mm</td>
<td>520056</td>
</tr>
<tr>
<td>Sample Holder</td>
<td>Holder 12x24 Tube</td>
<td>500199</td>
</tr>
<tr>
<td>Focused Ultrasonicator</td>
<td>Covaris S220x</td>
<td>S220x</td>
</tr>
<tr>
<td>Sample</td>
<td>Hydrogenated Egg Lecithin (HEL)</td>
<td>Avanti Lipids</td>
</tr>
<tr>
<td></td>
<td>DSPE-PEG2000</td>
<td>Avanti Lipids</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
<td>Sigma</td>
</tr>
<tr>
<td>Buffer</td>
<td>0.1 M PBS</td>
<td>Sigma</td>
</tr>
</tbody>
</table>

Operating Conditions

1. Fill the tank with fresh deionized water to proper fill line. The S220x should be equipped with a graduated water level label. If the tank lacks this label, please contact Covaris. During treatments, the tube should be partially immersed in the water to ensure a good acoustic path from the AFA transducer.

2. Degas water for the recommended 30 minutes or more. To maintain degassed water, keep the pump continuously on during operation and sample processing. Do not turn the pump off.

3. Set the chiller to the proper temperature, as listed for “Water Bath Temperature” in Table 1.
Recommendations Specific for Liposome Formation

The Covaris AFA process is highly reproducible, however steps should be taken to ensure the best results. The bath water is employed to couple acoustic energy to the sample vessel, thus attention must be paid to the following water treatment attributes to obtain the best results:

1. **Purity**: When applying acoustics in rate-limited applications, foreign materials such as algae and particulates may scatter the high frequency focused acoustic beam. Bath water should be pure distilled or DI water, changed daily or cleansed by a Covaris Water Conditioning System.

2. **Degas Level**: Similarly, insufficient degas levels within the bath may result in poor acoustic coupling. System degas pumps should be run in advance of and during AFA treatments, as detailed in instrument User Manuals.

3. **Temperature**: Warmer temperatures promote less forceful collapse of acoustic cavities within the sample fluid. Bath temperature (as reported by SonoLAB software) should therefore be closely controlled and matched run-to-run and day-to-day. Employ the temperature alert feature in SonoLAB to warn of a failure to maintain control of bath temperature.

4. **Level**: Attention should be paid to maintaining a consistent water level, according to published protocols. If using a Covaris Water Conditioning System, check levels daily to restore water lost to evaporation.

In summary, when employing the Covaris AFA, control and verification of treatment attributes and water quality will reduce variance and promote consistent, satisfactory results.

Method

1. Set up the Covaris S220x at the appropriate temperature following the operating conditions above.

2. Weigh out 14 mg of HEL, 4.5 mg of DSPE-PEG2000, and 3.5 mg of cholesterol, and add the sample to the sample vessel. This forms a mixture with a molar ratio of 1.85:0.15:1.

3. Add 2 mL of 0.1 M PBS to the vessel, and cap the tube. Be careful not to introduce a bubble into the bottom of the tube. This may happen if the water is added too quickly.

   **CAUTION:** The bottom of the tube is in the acoustic field. Therefore, a bubble in the sample will deflect energy and induce variable results.

4. Carefully load the sample vessel into the appropriate holder, and insert the holder into the S220x instrument.

5. Initiate and Run process according to the operating conditions specified in Table 1.
Supplementary Data

Description

A Malvern Zetasizer – 90 NS-90 may be used to analyze the liposomes formed using the Covaris S220x. The sample prepared above will be utilized for analysis.

Supplies

Table 3. Supplementary Equipment List

<table>
<thead>
<tr>
<th>Part Name</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malvern Zetasizer ZS-90</td>
<td>ZEN3690</td>
</tr>
<tr>
<td>Sample Cuvettes</td>
<td>DTS0012</td>
</tr>
</tbody>
</table>

Method

1. Set up the Malvern Zetasizer according to its setup instructions.
2. Add 1 mL of deionized water to the cuvette.
3. Aliquot 1 mL of prepared Liposome sample into cuvette. The prepared sample is the sample prepared on the previous page after it has been treated with AFA. Cap the cuvette.
4. Shake the cuvette by hand until the sample is dispersed evenly in the cuvette without air bubbles.
5. Place in Zetasizer instrument and run Volume Distribution Analysis.

Typical Output Readings: Intensity

Figure 1. Size Distribution by Intensity
Z-average = 128 nm; polydispersity index = 0.271

**Typical Output Readings: Variation**

*Table 4. Variation in Results*

<table>
<thead>
<tr>
<th>Process</th>
<th>PDI</th>
<th>Z-average(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.361</td>
<td>121.1</td>
</tr>
<tr>
<td>2</td>
<td>0.339</td>
<td>133.3</td>
</tr>
<tr>
<td>3</td>
<td>0.271</td>
<td>128</td>
</tr>
<tr>
<td>Average</td>
<td>0.324</td>
<td>127.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.038</td>
<td>5.0</td>
</tr>
<tr>
<td>CV</td>
<td>11.8%</td>
<td>3.9%</td>
</tr>
</tbody>
</table>

*Figure 2. Variation in Liposome Formation Results*