Giant Unilamelar Vesicles – preparation protocol - Nanion Chips

Lipid stock solution:

- 2.5mg/ml DPhPC (1,2 Diphytanoyl-sn-Glycero-3-Phosphocholine) in Chloroform ($CHCl_3$)
- 1-2% stearylamine
- 1% fluorescent lipid (1,2- Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-(Lissamine Rhodamine B Sulfonyl)(Ammonium Salt) (Avanti)) – only if fluorescence microscope is used.

Sucrose stock solution:

- 300mM Sucrose in distilled water

Buffer solution:

- 150mM KCl, 20mM MES (any other buffer should be ok!), pH 5

Step 1:
On the electrodes system 1.5µl drops form lipid stock solution were added at 5 different places on each electrode. The lipid drops were separated at about 3-5mm distance. The electrodes system was placed under vacuum for 30 minutes.

Step 2:
The 300mM sucrose solution was adjusted to the osmolarity of the buffer stock solution by adding distilled water or by increasing sucrose concentration.

Step 3:
After lipid drying under vacuum the electrodes were put in sucrose solution. A voltage generator was attached to the electrodes system and the following voltage protocol was applied:

Sinusoidal wave function was applied:
- 20 minutes 10 Hz and 0.1V;
- 3 hours 10Hz and 2V;
- 5 minutes 0.1Hz and 2V.

Step 4:

The liposomes were observed under the fluorescence microscope. A nice distribution implies large unilamelar vesicles. If so, dialysis was performed. The liposomes stock (3-4ml) was placed into a small recipient closed with a 1nm extrusion filter. The liposome containing recipient was placed into a large reservoir filled with buffer. The system was let at room temperature with continuous stirring for about 6-10h.

Note:
The protocol was not extensively used. It worked with more then 50% rate of success. The basic steps are present but small improvements are required.

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