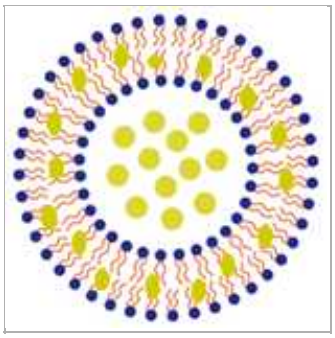

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G. Antimisiaris, S., 2016. Preparation of DRV Liposomes. [online] SpringerLink. Available at: [Accessed 25 October 2021].
Ahmad, H. and K. Dwivedi, A., 2017. Novel lipid nanostructures for delivery of natural agents with antioxidant, antiinflammatory and antistroke potential: perspectives and outcomes. [online] Science Direct. Available at: [Accessed 25 October 2021].
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Liposome Encapsulation of Hydrophilic and Hydrophobic Drugs

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Aisling Field

ABSTRACT

Dried reconstituted vesicles (DRV) are liposomes that are produced under mild conditions and can encapsulate very high amounts of hydrophilic solutes. These characteristics make this liposome type perfect for entrapment of drugs (Antimisiaris, 2016). To produce DRV's, the lipid ingredients are firstly dissolved in an organic solvent (chloroform), dried on a rotary evaporator to obtain a thin film, followed by lipid hydration (in the aqueous medium at a temperature greater than the phase-transition temperature) and lastly to homogenize and reduce the vesicle size, where the extrusion technique is used. This process produces heterogeneous multilamellar vesicles. Cholesterol is added into the bilayer structure to aid liposome formation, and to stabilize against aggregation and drug leakage (Ahmad and Dwivedi, 2017).

The thin-film method is one of the most widely used liposome encapsulation methods. It is based on the generation of a thin film of lipids, formed on the inner wall of the rotary evaporator flask. The film is then hydrated with water. Before the hydration, it is integral that the lipid film is preheated above the lipids transitional temperature to enable a smoother creation of the bilayer, along with the vigorous shaking. This allows the film to peel off the flask and form liposomes. The liposomes generated are multilamellar vesicles of different sizes. The encapsulating substance can be added with the lipids before the formation of the thin film (hydrophobic compounds) or with the water (hydrophilic compounds). The advantage of this method is its high reproducibility even when working with small quantities of compounds (Šturm andPoklarUlrih, 2021).

This protocol describes the liposome encapsulation of hydrophobic and hydrophilic drugs using thin-film dispersed hydration method. Extrusion method with a polycarbonate membrane is used to make liposomes of suitable sizes that can be easily internalized by mammalian cells.

Protocol status: Working
We use this protocol and it's working

Created: Dec 02, 2021

Last Modified: Jan 12, 2023

PROTOCOL integer ID: 55602

Keywords: liposome, hydrophilic, hydrophobic

MATERIALS

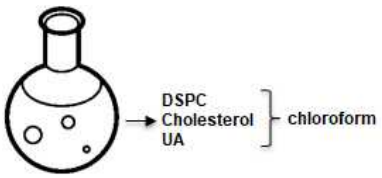
- Distearoylphosphatidylcholine (DSPC)
- Cholesterol
- Ursolic acid (UA)
- Chloroform (lab grade)
- Ultrapure water
- Round bottom flask
- Hot & stirring plate
- Magnetic stirrer
- Rotary Evaporator
- Incubator
- Avanti Extruder set
- PC membrane (0.05 nm and 0.1 nm)
- Vortex

SAFETY WARNINGS

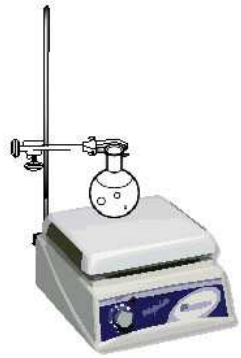
- Laboratory coat and gloves must be worn at all times
- Safety glasses must be worn when working with Chloroform in the fume hood

Liposome Encapsulation

1 Dissolve 7 mmol of the lipid (DSPC), 3 mmol of cholesterol and 1:20 (w/w) Ursolic acid (lipophilic compound) in 5 ml chloroform in a round bottom flask.



2 Stir the mixture for 15 min at above T_c of the lipid (60 ^{o}C).



3

Dry the lipid using a rotary evaporator at 40 ^{o}C.

4

Further dry overnight by incubating above the T_c of the lipid (60 ^{o}C) in a vacuum oven.

5

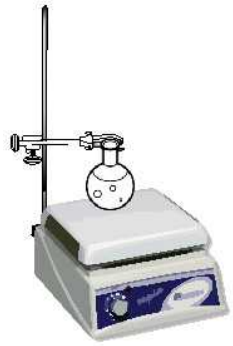
Re-hydrate lipid nanoparticle in 5 ml ultrapure water.

6

Liposome encapsulation of hydrophilic compounds are added after dehydration. Dissolve lipophilic compounds 1:20 (w/w) in 5 ml of ultrapure water. Re-hydrate lipid nanoparticles by adding the dissolved lipophilic compound in ultrapure water.

7

Stir the liposome encapsulated compound at a temperature above the Tc of the lipid (60 ^{o}C) for 30 min.



8

Vortex for 2 min.

9

Extrude at a temperature above the T_c of the lipid for approximately 22 passes using Avanti Mini Extruder Kit (Product Code: 610000). Do 11 passes with 100 nm pore size PC membrane and then another 11 passes using the 50 nm pore size PC membrane. This is done to have liposomes of between 50-100 nm in size.

Avanti Mini Extruder Procedure

10

Read particle size and zeta potential using Malvern Nano-ZS zetasizer.

11

A	B
Liposome	Concentrations
Liposome only	5.53mg DSPC, 1.16mg Cholesterol
Liposome-UA	0.35mg UA, 5.53mg DSPC, 1.16mg Cholesterol
Liposome-FITC	0.5ml FITC, 5.53mg DSPC, 1.16mg Cholesterol
Liposome-PI	0.5ml PI, 5.53mg DSPC, 1.16mg Cholesterol

Table 1. Liposome formulation

Liposome Characterisation

12 Turn on and warm up the Malvern Nano-ZS zetasizer for 1 hour for the laser to stabilize.

13 Open zetasizer software and set to the desired measurement.

Particle Size

14 Measure the particle size of liposome samples by setting a manual SOP.

15 Set the sample material to DTS0012 cuvette - polystyrene latex.

16 Set the dispersant of water to a temperature setting of 25 ºC.

17 Set the viscosity to 0.8872 cP.

18 Set the refracted index to 1.330.

19 Set the dielectric constant to 78.5.

20 Set the equilibration time to 120 seconds.

21 Set the measurement to 173º Backscatter.

22 Fill the cuvette with the sample to a depth of approximately 1 cm.

23 Place the cuvette with the sample in the cell of the zetasizer and analyse.

Zeta potential

24 Measure the zeta potential of liposome samples by setting a manual SOP.

25 Set the sample material to DTS1070 cuvette - polystyrene latex.

26 Set the dispersant water to a temperature setting of 25 ºC

27 Set the viscosity to 0.8872 cP.

28 Set the refracted index to 1.330.

29 Set the dielectric constant to 78.5.

30 Set the equilibration time to 120 seconds.

31 Set the measurement to 173º Backscatter and 100 zeta run.

32 Fill the cuvette with the sample to a depth of approximately 1 cm.

33 Place the cuvette with sample in the cell of the zetasizer and analyse.