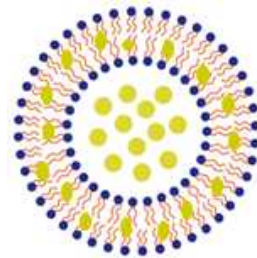


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Liposome Encapsulation of Hydrophilic and Hydrophobic Drugs

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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].
Šturm, L. and Poklar Ulrih, N., 2021. Basic Methods for Preparation of Liposomes and Studying Their Interactions with Different Compounds, with the Emphasis on Polyphenols. [online] mdpi. Available at: [Accessed 23 November 2021].

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Protocol status: Working

We use this protocol and it's working

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Keywords: liposome, hydrophilic, hydrophobic, liposome encapsulation method, used liposome encapsulation method, liposome encapsulation of hydrophilic, liposome encapsulation, liposome encapsulation of hydrophobic, form liposome, liposomes of suitable size, liposome type, liposome type perfect for entrapment, reconstituted vesicle, lipid film, vesicle, lipid ingredient, lipids before the formation, lipid hydration, heterogeneous multilamellar vesicle, multilamellar vesicle, lipid, multilamellar vesicles of different size, integral that the lipid film, encapsulating substance, vesicle size, hydrophobic drug, hydrophilic drug, lipids transitional temperature, hydrophilic solute, hydrophobic compound, hydrophilic compound, high amounts of hydrophilic solute, cholesterol, polycarbonate membrane, organic solvent, drv, thin film, bilayer structure, dispersed hydration method, hydration method, rotary evaporator flask, smoother creation of the bilayer, film method, drug leakage, inner wall of the rotary evaporator flask

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 (Antimisariis, 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 and PoklarUlrih, 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.

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

Troubleshooting



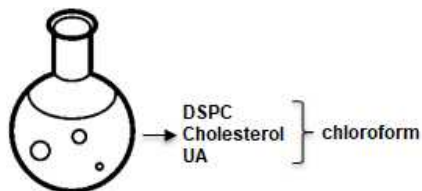
Safety warnings

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

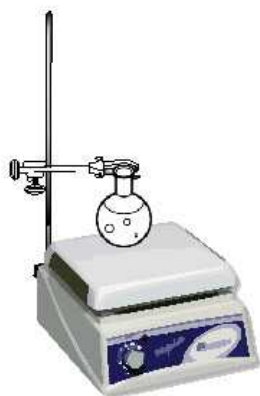
Liposome Encapsulation

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 °C).

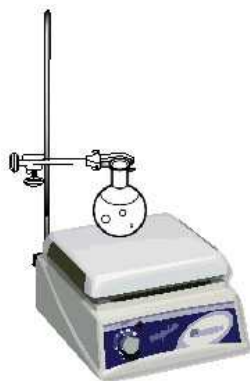


3

Dry the lipid using a rotary evaporator at 40 °C.



- 4
Further dry overnight by incubating above the T_c of the lipid (60 °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 T_c of the lipid (60 °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 °C.
- 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 173° 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 °C
- 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 173° 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.

