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Preparation of fatty acid or phospholipid vesicles by thin-film rehydration

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Purpose

Preparation of polydisperse, multilamellar vesicles through the rehydration of a thin film of fatty acids or phospholipids.

Theory

The rehydration of a dry film of lipid(s) leads to the formation of vesicles. The lipid composition for the membranes can include phospholipids, single chain lipids (fatty acids, glycerol esters), sterols, or mixtures of various amphiphiles. For fatty acid vesicles, the buffer pH should be near the pKa of the bilayer-associated fatty acid [1]. The encapsulated contents of the vesicles are determined by the buffer used for the rehydration.

Equipment

Rotary evaporator

Glass 10 ml round-bottom flask with cap

Bench top rotary tumbler

Bench top vortex machine

pH meter

1.5 ml Eppendorf tubes

Materials

1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC)

Lissamine™rhodamine B 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Rh-DHPE)

Oleic acid

Myristoleic acid

Glycerol monomyristoleate (GMM)

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Bicine (or other buffer of choice, except borate or phosphate buffer, which produces leaky fatty acid vesicles)

8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS, or other water-soluble fluorescent dye of choice)

NaOH

Chloroform

Methanol

Deionized water

Solutions & buffers

Preparation—20 mM POPC, 10 mM oleic acid in chloroform

Component	Stock	Amount
20 mM POPC in chloroform	20 mM	1 ml
oleic acid (pure)	>99%	3.2 ul

10 mM oleic acid, 0.1 mM Rh-DHPE in chloroform

Component	Stock	Amount
chloroform	pure	1 ml
oleic acid (pure)	>99%	3.2 ul
Rh-DHPE in chloroform	10 mM	10 ul

20 mM myristoleic acid, 10 mM glycerol monomyristoleate in chloroform (or use methanol)

Component	Stock	Amount
chloroform	pure	1 ml
myristoleic acid (pure)	>99%	5.6 ul
glycerol monomyristoleate (pure)	>99%	2.8 ul

Step 2—Na-bicine buffer (200 mM), 2 mM HPTS, pH 8.5

Component	Stock	Amount
Na-bicine	1 M	1 ml
HPTS	100 mM	0.1 ml

Add water to 5 ml

Na-bicine buffer (200 mM), pH 8.5

Component	Stock	Amount
Na-bicine	1 M	1 ml

Add water to 5 ml

Protocol

Duration

Preparation	about 10 minutes
Protocol	about 24 hour

Preparation

Prepare a solution containing the desired lipid composition for vesicles in a non-polar solvent (e.g. chloroform).

Caution

Work in a hood. All lipids should be stored at −20°C. Always use glass tips for pipetting chloroform.

Step 1 Formation of a thin lipid film

Overview

Formation of a thin layer of dry lipid film in a round-bottom flask.

Duration

30 min

1.1 Pipette the prepared solution of the desired lipids in a non-polar solvent into a 10 ml round-bottom flask. If fatty acid(s) are in the desired lipid composition, pipette the appropriate amount of pure fatty acid into the round-bottom flask first (see Fig. 1)

Tip

Clean the round-bottom flask with methanol before the procedure.

Tip

Avoid light by wrapping aluminum foil around the sample. Avoid oxygen by flushing the container with argon or nitrogen gas.

1.2 Rotary evaporate the round-bottom flask to completely eliminate the chloroform in the sample (see Fig. 2). Alternatively, dry the film under a stream of argon while manually rotating the flask (see Fig. 3).

Tip

To ensure all solvent is removed from the film, leave the flask under vacuum for 1 hour.

Tip

If only fatty acids or glycerol esters are in the desired lipid composition, one can skip the step of desolving fatty acids into chloroform, and instead directly add neat fatty acids or glycerol esters to the buffer solution to make vesicles [2].

Step 2 Rehydration of the thin lipid film

Overview

Rehydration of the thin lipid film by adding buffer solution, leading to the formation of vesicles.

Duration

20 min

2.1 Add the prepared buffer solution to the round-bottom flask. Any solutes to be encapsulated should be included in the buffer.

2.2 Tightly cap the round-bottom flask, briefly vortex, and tumble for 10 min, until the thin lipid film at the bottom of the flask is completely dispersed in the buffer (see Fig. 4).

2.3 Pipette the sample into a 1.5 ml Eppendorf tube, vortex briefly, and tumble overnight (see Fig. 5).

Tip

Multiple cycles of freezeing and thawing the vesicle sample may improve the encapsulation efficiency.

Tip

A thin film of phospholipid(s), does not desolve well in a buffer solution without any metal ions (e.g., ammonium acetate solution without Na^+). In this case, adding a small amount of NaCl or NaOH helps to desolve the lipid.

Source article(s) used to create this protocol

1. Chen IA, Roberts RW, Szostak JW. The emergence of competition between model protocells. Science. 2004 Sep 3; 305(5689):1474–6. [PubMed: 15353806]
2. Hanczyc MM, Fujikawa SM, Szostak JW. Experimental models of primitive cellular compartments: encapsulation, growth, and division. Science. 2003 Oct 24; 302(5645):618–22. [PubMed: 14576428]

Referenced literature

1. Cistola DP, Hamilton JA, Jackson D, Small DM. Ionization and phase behavior of fatty acids in water: application of the Gibbs phase rule. Biochemistry. 1988; 27:1881–88. [PubMed: 3378036]

2. Hanczyc MM, Fujikawa SM, Szostak JW. Experimental models of primitive cellular compartments: encapsulation, growth, and division. Science. 2003 Oct 24; 302(5645):618–22. [PubMed: 14576428]

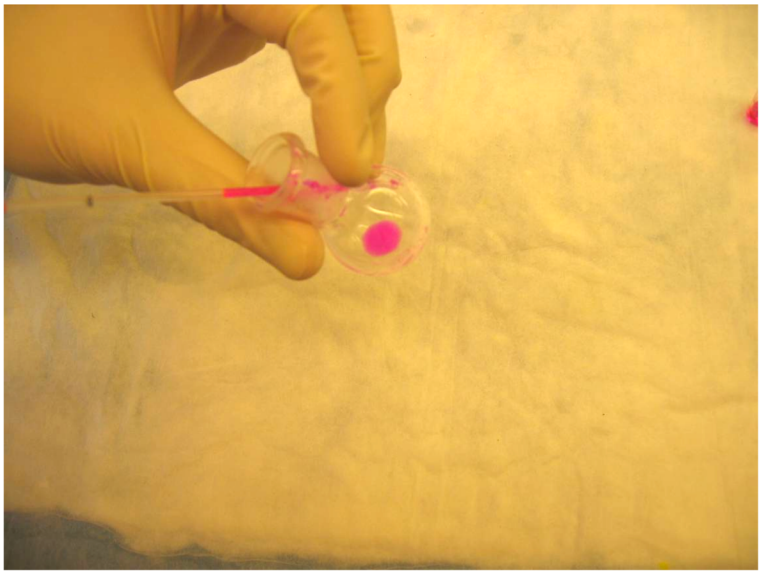

Fig. 1.
Pipette the prepared solution of the desired lipids in a non-polar solvent into the round-bottom flask.

Fig. 2.
Remove chloroform by rotary evaporation.

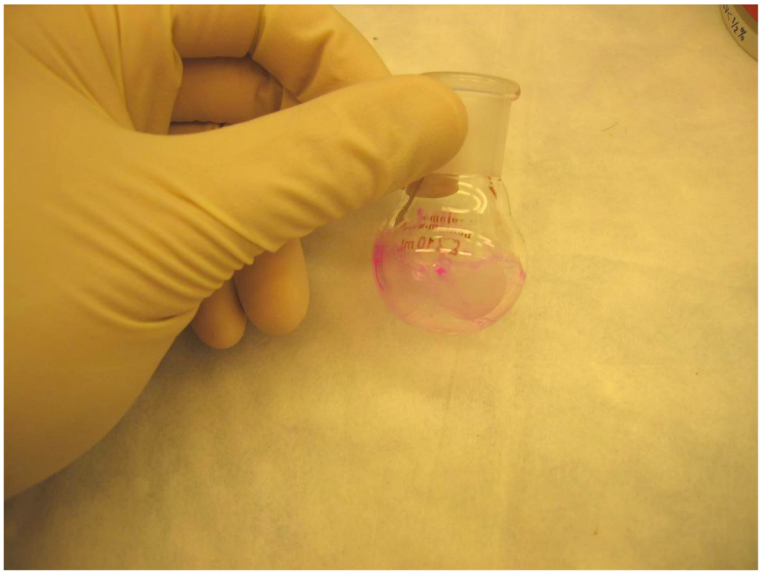

Fig. 3.
Formation of a dry lipid film in a round-bottom flask.

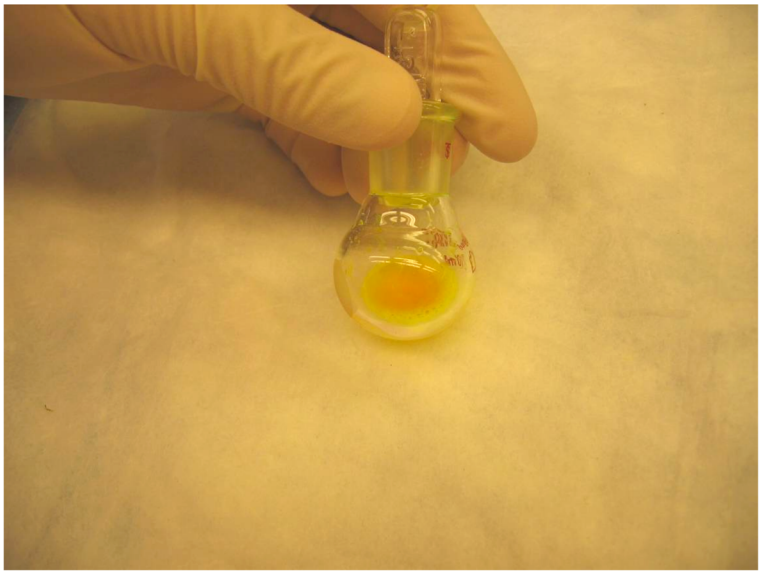

Fig. 4.

The thin lipid film (red) at the bottom of the flask is completely dispersed in the buffer containing 2 mM HPTS (green).

Fig. 5.
Vesicle suspension in a 1.5 ml Eppendorf tube, on a bench top rotary tumbler.