CHAPTER TWENTY

# Preparation of Fatty Acid or Phospholipid Vesicles by Thin-film Rehydration

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## Contents



## Abstract

Prepare polydisperse, multilamellar vesicles by rehydrating a thin film of fatty acids or phospholipids.

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## 1. 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 (Cistola et al., 1988). The encapsulated contents of the vesicles are determined by the buffer used for the rehydration.

## 2. EQUIPMENT

Rotary evaporator
10-ml round-bottom glass flasks with cap
Benchtop rotary tumbler
Vortex mixer
pH meter
Glass pipettes
1.5-ml microcentrifuge tubes

## 3. 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)
Bicine
8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS)
Sodium hydroxide (NaOH)
Chloroform
Methanol
Deionized water

### 3.1. 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 | >99% | 3.2 μl |

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

| Component | Stock | Amount |
|---|---|---|
| Chloroform | | 1 ml |
| Oleic acid | >99% | 3.2 µl |
| Rh-DHPE in chloroform | 10 mM | 10 µl |

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

| Component | Stock | Amount |
|---|---|---|
| Chloroform | | 1 ml |
| Myristoleic acid | >99% | 5.6 µl |
| Glycerol monomyristoleate | >99% | 2.8 µl |

**Step 2** Na-bicine buffer with HPTS, pH 8.5

| Component | Final concentration | Stock | Amount |
|---|---|---|---|
| Na-bicine | 200 mM | 1 M | 1 ml |
| HPTS | 2 mM | 100 mM | 0.1 ml |

Add water to 5 ml

Na-bicine buffer, pH 8.5

| Component | Final concentration | Stock | Amount |
|---|---|---|---|
| Na-bicine | 200 mM | 1 M | 1 ml |

Add water to 5 ml

# 4. PROTOCOL

## 4.1. Duration

| Preparation | About 10 min |
|---|---|
| Protocol | About 24 h |

See Fig. 20.1 for an overview of the complete protocol.

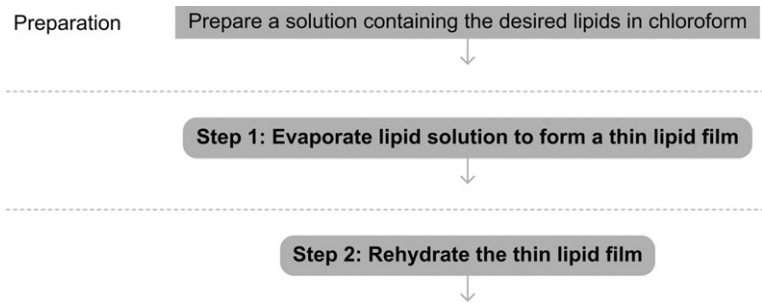


**Figure 20.1** Flowchart of the complete protocol, including preparation.

### 4.2. Preparation

Prepare a solution containing the desired lipid composition for vesicles in a nonpolar solvent (e.g., chloroform).

### 4.3. Caution

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

See Fig. 20.1 for the flowchart of the complete protocol.

## 5. STEP 1 FORMATION OF A THIN LIPID FILM

### 5.1. Overview

Evaporate a solution of lipids to form a thin layer of dry lipid film in a round-bottom flask.

### 5.2. Duration

30 min

**1.1** Pipette the prepared solution of the desired lipids in a nonpolar 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. 20.2).

**1.2** >Rotary evaporate the lipids in the round-bottom flask to completely eliminate the chloroform in the sample (see Fig. 20.3). Alternatively, dry the film under a stream of argon while manually rotating the flask (see Fig. 20.4).

### 5.3. Tip

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

**Figure 20.2** Pipette the prepared solution of the desired lipids in a nonpolar solvent into the round-bottom flask.

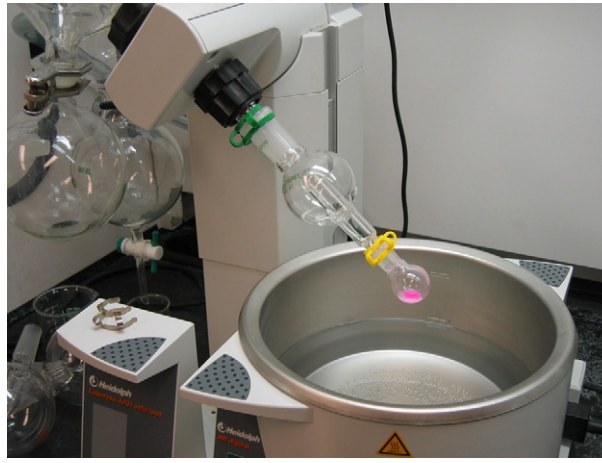

**Figure 20.3** Remove chloroform by rotary evaporation.

## 5.4. Tip

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

## 5.5. Tip

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

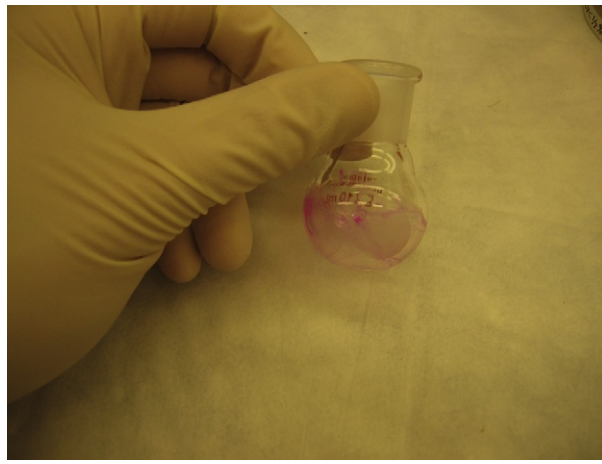

**Figure 20.4** Formation of a dry lipid film in a round-bottom flask.

### 5.6. 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 (Hanczyc et al., 2003).*

## 6. STEP 2 REHYDRATION OF THE THIN LIPID FILM

### 6.1. Overview

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

### 6.2. Duration

20 min

**2.1** Add the prepared buffer solution to the round-bottom flask. Any solutes to be encapsulated in the vesicles 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. 20.5).

**2.3** Pipette the sample into a 1.5-ml microcentrifuge tube, vortex briefly, and tumble overnight (see Fig. 20.6).

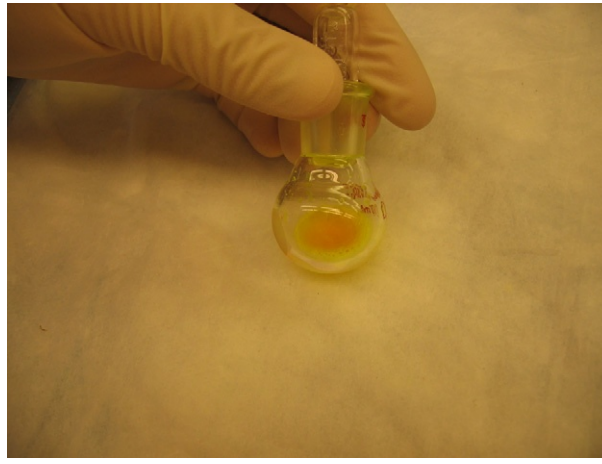

**Figure 20.5** The thin lipid film (red) at the bottom of the flask is completely dispersed in the buffer containing 2 mM HPTS (green).

**Figure 20.6** Vesicle suspension in a 1.5-ml microcentrifuge tube, on a bench top rotary tumbler.

## 6.3. Tip

*Do not use borate or phosphate buffers, since they produce leaky fatty acid vesicles.*

## 6.4. Tip

*You can use a water-soluble fluorescent dye other than HPTS.*

### 6.5. Tip

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

### 6.6. Tip

*A thin film of phospholipid(s) does not dissolve 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 dissolve the lipid.*

## REFERENCES

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