Preparation of lipid films for phospholipid liposomes

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Abstract

This protocol (like its companion protocol for liposome suspension and extrusion) was original created by Krista Longnecker and Jamie Collins for creating lipsomes to be used in lipid photo-oxidation experiments. The results of these experiments are detailed in Chapter 4 of:

Collins, J. R. 2017. The remineralization of marine organic matter by diverse biological and abiotic processes. Ph.D. thesis. Cambridge, Massachusetts: Massachusetts Institute of Technology, 300 pp; doi:10.1575/1912/8721

A manuscript of the chapter is forthcoming.

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Protocol

Obtain desired lipids from supplier

Step 1.

For this work, we used a variety of phosopholipids obtained from Avanti Polar Lipids, Inc. Where available, we obtained lipids as a powder. When the desied lipid was not available from the supplier as a powder (e.g., 22:6, 22:6 PC), we ordered the product dissolved in chloroform. The lipids given in the reagents list for this step were those used for the lipid photo-oxidation experiments, but this protocol can be followed for preparation of films of any phospholipid.

REAGENTS

1,2-dioleoyl-sn-glycero-3-phosphocholine (18:1, 18:1 PC) 850375P by Avanti Polar Lipids, Inc.

1,2-dipalmitoyl-sn-glycero-3-phosphocholine (16:0, 16:0 PC) 850355P by Avanti Polar Lipids, Inc.

1,2-dipalmitoleoyl-sn-glycero-3-phosphocholine (16:1, 16:1 PC) 850358P by Avanti Polar Lipids, Inc.

1,2-distearoyl-sn-glycero-3-phosphocholine (18:0, 18:0 PC) 850365P by Avanti Polar Lipids, Inc.

1,2-dilinoleoyl-sn-glycero-3-phosphocholine (18:2, 18:2 PC) 850385P by Avanti Polar Lipids, Inc.

1,2-dibehenoyl-sn-glycero-3-phosphocholine (22:0, 22:0 PC) 850371P by Avanti Polar Lipids, Inc.

1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine (22:6, 22:6 PC) 850400C by Avanti Polar Lipids, Inc.

Dissolve lipids in chloroform

Step 2.

In a fume hood, add 200 µL of chloroform to each glass test tube to dissolve the lipids. Just add enough chloroform that the lipids dissolve. The actual volume of chloroform does not matter because it will be removed in a later step.

Note that this step **does not apply** if lipid was obtained from vendor already in chloroform.

If making multiple films of each lipid: Add more chloroform to this first tube — 200 µL × no. of desired films — and then aliquot the solution into separate tubes in volumes of 200 µL.

REAGENTS

Chloroform by Contributed by users

Disposable borosilicate glass culture tubes, with rounded bottom 14-961-26 by Fisher Scientific

SAFETY INFORMATION

Chloroform is a hazardous solvent. Conduct all work in a fume hood, while wearing proper PPE.

Weigh out desired quantity of lipid(s) into a glass test tube

Step 3.

Weigh out desired quantity of lipid(s) into a glass test tube. Use a round-bottom tube, such a disposable culture tube or small (i.e., 20 mL) round-bottom vial from an HPLC fraction collector. In applying this protocol, we aimed for approx. 1.65 mg of each lipid per tube. If making up multiple tubes of lipid fims for each lipid, weigh out the total quantity of lipid needed (1.65 × no. of tubes) into a single tube; you can then aliquot it into separate tubes using a solvent-rinsed glass syringe.

Optional: If precise concentration of lipid in initial chloroform solution must be known

Step 4.

If you want to know the exact concentration of lipids in any of these initial chloroform solutions, remove an aliquot of the lipid/chloroform solution at this point, and keep track of the volume. The lipid solution will need to be diluted further with chloroform before it can be analyzed. Helen Fredricks (HPLC-MS guru in the Van Mooy Lab) has suggested a final concentration of 0.01 mg mL^{-1} for analysis.

Optional: Dispense ~ 200 µL aliquots into different tubes

Step 5.

If you made up more than 200 µL of lipid solution in your initial tube, now is the time to dispense 200 µL aliquots into separate round-bottom tubes. A volumetric, solvent-washed glass syringe can be used

for this purpose.

Blow down the lipid/chloroform solution in each tube using a nitrogen gas blow-down system

Step 6.

The object here is to create a lipid 'film' that evenly coats the inside of the lower side walls and bottom of each tube once the solvent has been evaporated. This requires some concentration and attention when evaporating the solvent.

You cannot just stick the vial under a needle with N_2 flowing out of it and walk away, because that will not form the lipid film needed for this method to work.

Best practices:

1. Connect a disposable glass pasteur pipette (use one with a longer length) to the nitrogen supply by way of some flexible non-reactive (e.g., Tygon) tubing. Use some lab tape or other means to secure the pipet at about eye height inside the fume hood. You will want the end of the pipette to point slightly downward. The precise orientation of the pipette doesn't matter all that much; you want it oriented in such a way to make the next few steps convenient and easy!
2. Hold a tube containing 200 µL of the lipid solution so that the end of the pipette protrudes just into the mouth of the tube.
3. While contantly rolling the tube in your fingers, gradually let a weak stream of the N_2 blow down the chloroform. Remember: You don't want to concentrate all the lipid at the very bottom of the tube. The goal is to create a film that evenly and continuously coats the lower third of the side wall and bottom of the tube.
4. At the end, you should have an even film of the lipid coating the lower side walls and bottom of the tube. If you end up with any chunks or visible crystals, add chloroform and repeat the film-coating and blowdown process again.

REAGENTS

- Glass pasteur pipettes by Contributed by users
- Nitrogen gas, UHP by Contributed by users

Remove any residual chloroform

Step 7.

Once the vial appears dry, remove any residual chloroform by putting the tubes in a Speed Vac (or Vacufuge) for one hour.

SAFETY INFORMATION

Note: Make sure you use a vacuum evaporator and tubing suitable for work with organic solvents.

Top fims with argon and store until needed

Step 8.

Top the dry lipid film with argon (as a stabilizer), cap, and then and store at −20ºC until needed.

We have stored the films for up to 4 months when argonized and capped.

REAGENTS

- Argon gas, UHP by Contributed by users