Morrissey Lab Protocol for Preparing Phospholipid Vesicles (SUV) by Sonication

Protocol from James H. Morrissey , Dept. of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Background:

In this method, phospholipids are first suspended in a buffered saline solution to give large, multilamellar vesicles. The vesicle suspension is then sonicated to break up the phospholipid suspension into small, unilamellar vesicles (SUV).

To sonicate the vesicle suspension, we use a bath sonicator (Model G112SPIG from Laboratory Supplies Co., Inc., 29 Jefry Lane, Hicksville, NY 11801; Tel: 516-681-7711). This sonicator is inexpensive, works very well for making vesicles, and does not generally require the user to wear hearing protection while operating it. The Avanti web site has more information on this sonicator.

We use these vesicles in our research on blood clotting.

Alternative procedures to make uniform, unilamellar vesicles include membrane extrusion and detergent dialysis .

Method:

1. Dispense 2.6 µmole total phospholipids (PL) in a *glass* test tube (a 13 x 100 mm tube is a convenient size).

Examples*—amounts of PL to use in making PCPS or PCPSPE vesicles:*

For PC:PS vesicles (80:20 molar ratio)		
63 µ**L PC** (at 25 mg/ml) (or **158** µ**L** at 10 mg/ml)	= 1.58 mg	= 2.08 µmole
42 µ**L PS** (at 10 mg/ml)	= 0.42 mg	= 0.52 µmole

For PC:PE:PS vesicles (40:40:20 molar ratio)		
32 µ**L PC** (at 25 mg/ml) (or **79** µ**L** at 10 mg/ml)	= 0.79 mg	= 1.04 µmole
80 µ**L PE** (at 10 mg/ml)	= 0.80 mg	= 1.04 µmole
42 µ**L PS** (at 10 mg/ml)	= 0.42 mg	= 0.52 µmole

Be sure to overlay the contents of the stock vials of phospholipid with argon gas before capping and returning them to the freezer!

2. In the fume hood, dry the PL mixture under a gentle stream of nitrogen or argon. When dry, speed-vac for an additional 60 minutes under high vacuum. (This is to remove any residual chloroform.)

3. To the dried-down PL, add 2.6 ml room temperature HBS solution and cover the end of the tube with parafilm. Let sit 1 hr at room temperature.

4. Vortex tube vigorously to completely resuspend the PL. The result should be a milky, uniform suspension.

5. Fill the bath sonicator with room temperature water. Using a ring stand and test tube clamp, suspend the tube containing the PL suspension in the bath. The liquid level inside the tube should equal that outside the tube. Sonicate until the suspension changes from milky to nearly clear (i.e., only very slightly hazy) in appearance. Check every 10 min; it will usually take between 10 and 30 min total sonication time. (Be sure not to let the bath overheat, and do not drain the bath until it has completely cooled.)

6. Store the final product at 4 degrees C. The result is a suspension of small unilamellar vesicles (SUV) containing a total of 1 mM phospholipid in HBS.

Notes:

You can confirm the phospholipid concentration by assaying total phosphorus content . See the Avanti web site for more information on, and discussions about, the preparation of phospholipid vesicles.

The method can be easily scaled up. Simply make a larger batch, and sonicate in a larger test tube (the sonication time may be longer). If you want to make very large amounts, sonicate in small batches.

Materials and Solutions:

HBS (store at room temperature)
100 mM NaCl 20 mM Hepes/NaOH buffer, pH 7.5 0.02% (w/v) sodium azide

Phospholipid Stock Solutions*				
	Phospholipid name	*concentration*	*MW*	*cat. #*
PC	L-alpha-Phosphatidylcholine, egg	10 or 25 mg/ml	761	840051
PS	L-alpha-Phosphatidylserine, bovine brain-Na salt	10 mg/ml	810	840032
PE	L-alpha-Phosphatidylethanolamine, bovine liver	10 mg/ml	768	840026

*We buy our phospholipids from Avanti Polar Lipids , dissolved in chloroform. The stock solutions should be stored at -20 degrees C under argon. Do not store more than 3 months (6 months for PC).