

Sizing of Liposomes by TEM

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We are using negative stain or a combination of osmium tetroxide fixation and negative stain to monitor the size homogeneity of lipid vesicle/liposome populations prepared by such techniques as sonication, extrusion, and gel filtration chromatography. Small sonicated vesicles up to about 50 nm in diameter seem sufficiently well preserved in negative stain for adequate size determination, although some flattening upon adherence probably does occur. Larger vesicles such as 100 nm extruded liposomes which contain a large aqueous core are likely to be flattened significantly in negative stain and may be partially stabilized with an osmium tetroxide fixation. The effectiveness of this fixation will in large part depend upon the degree of saturation of the lecithin species.

Osmium fixation has been extremely valuable in the preparation and sizing of synthetic emulsions composed of triolein, egg yolk phosphatidylcholine (EYPC), and cholesterol and lipoproteins such as very low density lipoprotein (VLDL) and chylomicrons. This works by minimizing the flattening of these fixed particles as verified by unidirectional shadowing. Although we have not shadowed fixed vesicles, a quick look at a tilted image in the TEM is useful to detect major flattening.

The fixation/negative staining procedure is based upon the widely used drop technique:

- 1) Add 1 volume of 2% OsO₄ in cacodylate buffer (phosphate buffer or others can be used) to 2 volumes of the lipid preparation. Fix for at least 30 minutes.
- 2) Place a small aliquot of fixed suspension on a freshly

glow-discharged carbon and formvar-coated grid for a few seconds.

- 3) Remove and blot off excess fluid and immediately stain with 1% sodium phosphotungstate titrated to appropriate pH with 1 N NaOH.

- 4) After a few seconds, blot excess fluid and air dry.

The final concentration of particles on the grid can be adjusted by diluting the fixed material with water or varying the incubation time on the grid. If sample staining is complicated by high salt content or buffer/stain incompatibility, the grid can be rinsed with several drops of water after sample incubation and before negative staining. In addition, floating an inverted grid upon the droplet of sample may result in a cleaner background.

If lamellar structure of vesicle/liposome preparations is of interest, other techniques such as vitreous ice cryomicroscopy and freeze fracture should be considered, since in our experience negative stain preparation is quite variable. For example, pure EYPC sonicated vesicles are generally unilamellar; and extruded mixtures of EYPC and cholesterol tend to be a mix of uni- and multilamellar vesicles.

Although cryomicroscopy (a method that we have available) provides very useful information on lamellar structure and polymorphism, size distribution may not be representative. A sizing method complementary to negative staining such as dynamic light scattering is also useful. ■



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