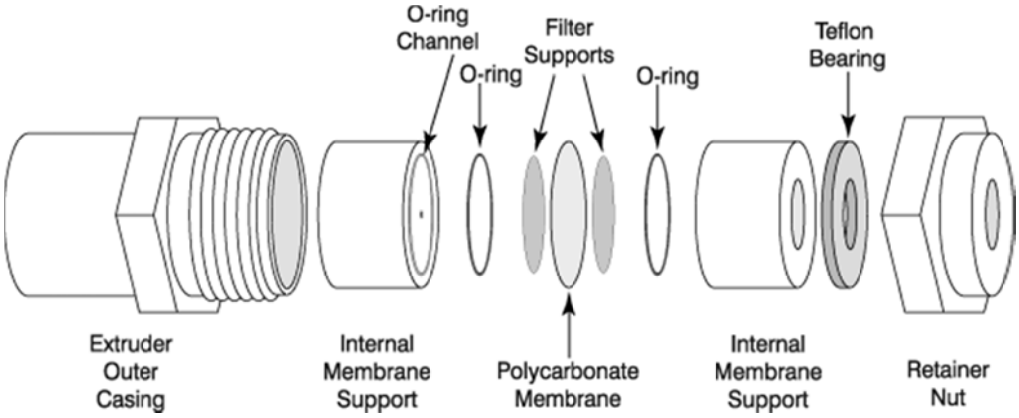
Assembly Instructions

Before assembling the extruder for the first time, thoroughly clean all parts (except for the Polycarbonate Membranes and the Filter Supports) with a mild detergent solution, followed by rinsing with hot tap water and deionized or distilled water. Allow all parts to dry before assembling apparatus.

Important Note: The original Mini-Extruder design contains flat washers, and a bearing washer, that is not stainless steel and will rust if not thoroughly dried. Rinse with methanol or acetone and dry immediately. The all-teflon Mini-Extruder design replaces these components with a teflon bearing. The teflon bearing is available as a replacement; part number 610019, Teflon washer for extruder

Assembly - refer to the diagram to identify the parts in these instructions.



1) Place the 2 Internal Membrane Supports on a flat surface with the O-rings facing up.

2) Pre-wet 2 Filter Supports with DI water, or buffer, and place over orifice. The Filter Supports should adhere to the teflon orifice inside the O-ring inner diameter.

3) Insert the Internal Membrane Support, with the Filter Support, into the Extruder Outer Casing with the O-ring facing up.

4) Place 1 Polycarbonate Membrane in the Extruder Outer Casing over the Filter Support and O-ring.

Note: The Polycarbonate Membrane is the thin, shiny disk - do not install one of the blue paper disks which separate the Polycarbonate Membranes

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5) Pre-wet a second pair of Filter Supports with DI water, or buffer, and place over orifice of remaining Internal Membrane Support.

6) Carefully place the second Internal Membrane Support into the casing (O-ring facing down) being careful not to twist the Membrane Support when it comes in contact with the Membrane.

7) If you have the original Mini-Extruder design, place the flat washers on either side of the bearing washer and insert into the Retainer Nut. For the all-teflon Mini-Extruder, place the teflon bearing into the Retainer Nut.

8) Place the Retainer Nut on the threaded end of the Extruder Outer Casing and tighten. **Tighten the Retainer Nut by hand just until it is finger tight; do not use a wrench.**

Important Note: Autoclaving the Teflon inserts is not recommended as slight distortion may occur (~0.002mm). The use of ethylene oxide or gamma irradiation is suggested as an alternative. The syringes should be sterilized with ethanol.

Extrusion Technique

Note: Extrusion of multilamellar liposomal suspensions using membranes with a pore size >0.2µm does not produce unilamellar liposomes. Liposomes produced with larger pore membranes will yield a polydisperse suspension of multilamellar liposomes. Unilamellar liposomal suspensions with a low polydispersity can only be prepared with membranes having a pore size of ≤0.2µm.

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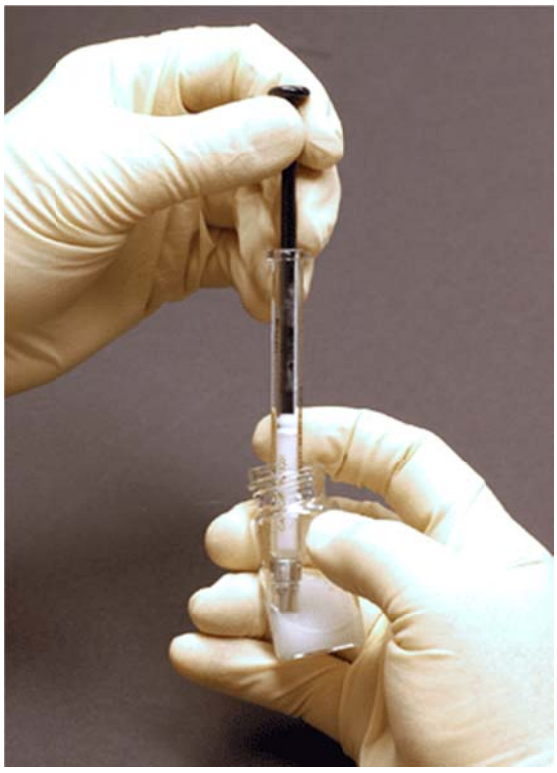
1) Prepare dry lipid mixture by lyophilization or evaporation.

2) Place the extruder stand / heating block onto a hot plate. Insert a thermometer into the well provided in the heating block. Switch the hot plate on, and allow to reach the desired temperature. Allow the temperature of the heating block to reach the desired value (approximately 15 minutes).

3) Hydrate lipid mixture using a suitable buffer for at at least 30 min. The lipid suspension should be kept above the phase transition temperature of the lipid during hydration and extrusion. To increase the efficiency of entrapment of water-soluble compounds, one may subject the hydrated lipid suspension to 3-5 freeze/thaw cycles by alternately placing the sample vial in a dry ice bath and warm water bath.

4) Once the sample is fully hydrated, load the sample into one of the gas-tight syringes and carefully place into one end of the mini-extruder.

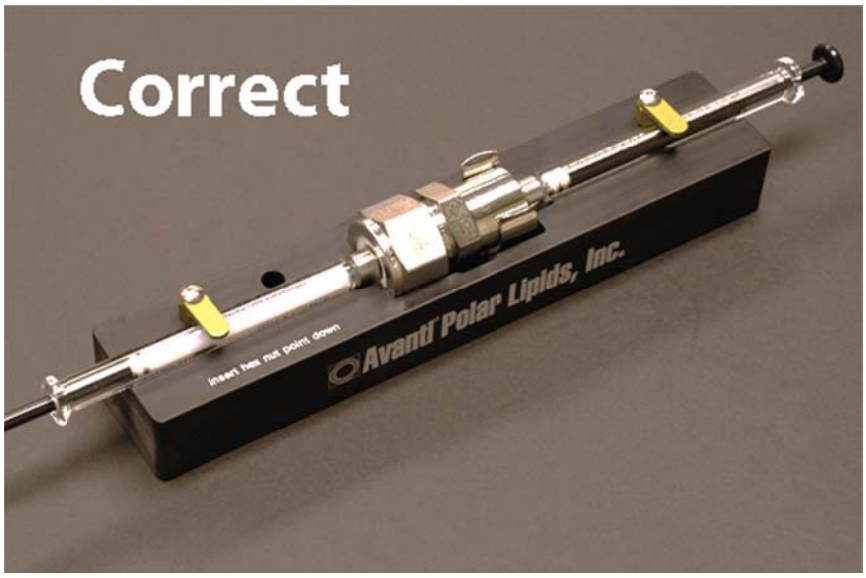


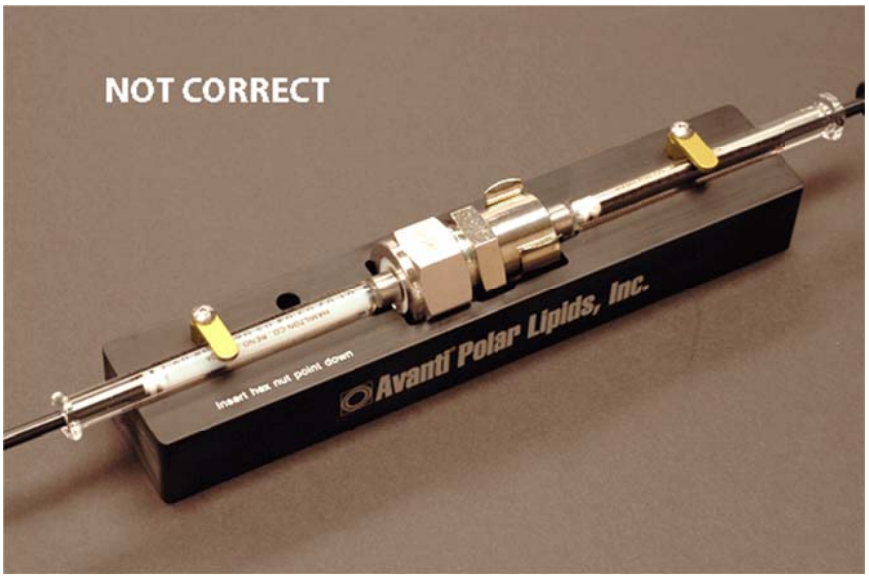
Note: To reduce the dead volume, pre-wet the extruder parts by passing a syringe full of buffer through the extruder; discard the buffer. New syringes may have tight fitting parts; to facilitate extrusion, pre-wet syringe barrel and plunger with buffer prior to inserting plunger into barrel.

5) Place the empty gas-tight syringe into the other end of the mini-extruder. Make sure the empty syringe plunger is set to zero; the syringe will fill automatically as the lipid is extruded through the membrane.

6) Check the temperature of the heating block BEFORE placing the assembled extruder apparatus into the heating block. The temperature must be below 80°C, or the syringes will be damaged.

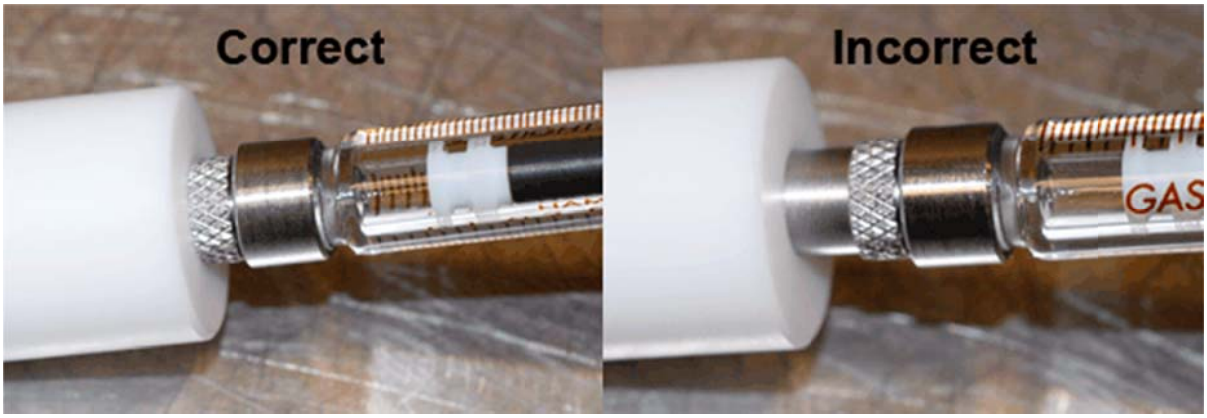
7) Insert the fully assembled extruder apparatus into the extruder stand. Insert the hex nut so that the apex of the hex nut points down.





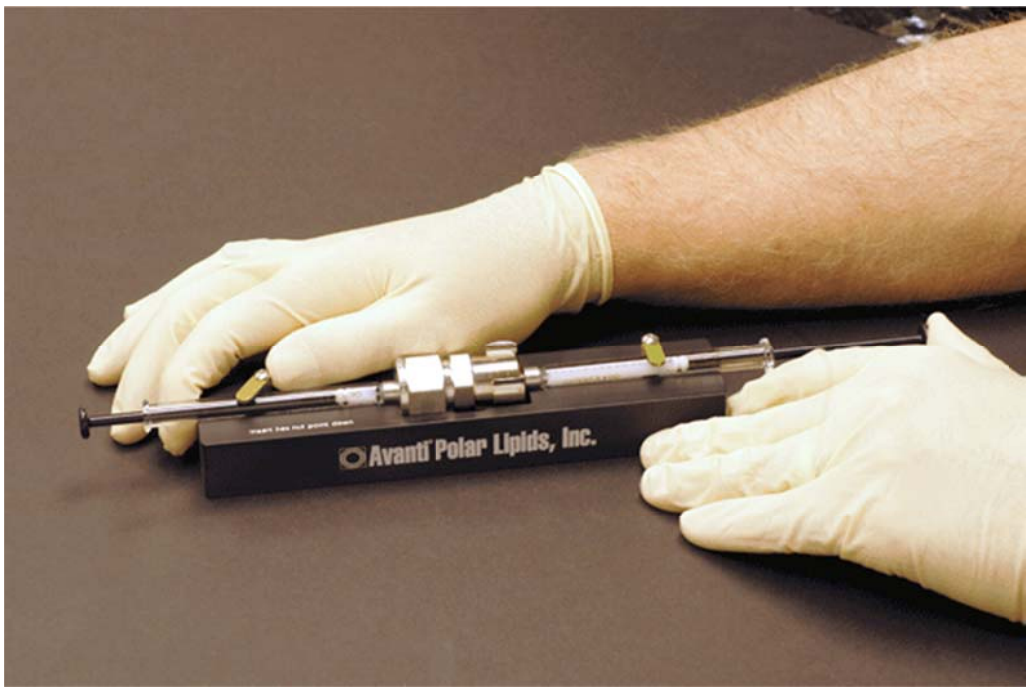
Use the swing-arm clips to hold the syringes in good thermal contact with the heating block.

Note: The extruder apparatus must be fully assembled before inserting in the heating block, otherwise it will be damaged. The Syringes should fit tight into the Extruder.



8) Allow the temperature of the lipid suspension to equilibrate with the temperature of the heating block (approximately 5-10 minutes)

9) Gently push the plunger of the filled syringe until the lipid solution is completely transferred to the alternate syringe.



10) Gently push the plunger of the alternate syringe to transfer the solution back to the original syringe.

11) Repeat previous two steps. A minimum of 4 times (total of 10 passes through membrane). In general, the more passes though the membrane, the more homogenous the lipid solution becomes.

12) The final extrusion should fill the alternate syringe. This is to reduce the chances of contamination with larger particles or foreign material.

13) After the final extrusion, remove the mini-extruder from the heating block.

14) Remove the filled syringe from the extruder and inject the lipid solution into a clean sample vial. Important: When removing syringes, pull the syringe straight out of the extruder; removing at an angle could result in cracking the syringe.

15) Store the vesicle preparation above the transition temperature of the lipid during the experiment. When not in use, store the vesicle solution at 4°C. Do not freeze. Vesicle solutions are not stable in aqueous media for more than 3-4 days when stored at 4°C. Storage of vesicle solutions at higher temperatures and pH <5 or >8 may reduce the lifetime of the vesicle suspension.

16) Clean apparatus thoroughly before using with a new lipid preparation.

Care and Cleaning Instructions for Syringes

1) Immediately after use, carefully disassemble both syringes.

2) Depending upon your application, rinse the syringes with either isopropyl alcohol, DI water, or a weak detergent solution. If a detergent solution is used, rinse the syringe immediately with copious quantities of DI water.

3) Rinse the syringes with DI water after cleaning.

4) Dry the syringes.

5) If it is necessary to use a sonic cleaner, only clean the needle with the sonic cleaner. Immediately after cleaning in the sonic cleaner, rinse with DI water and dry.

6) The Plunger Assembly may need to be replaced if the syringes are used at elevated temperatures for extended periods of time. Replacement parts are available here.

Important Note: Do not allow the syringes to come into contact with any solvents other than DI water or alcohol. Some organic solvents will interact with the glue holding the threaded insert onto the tip of the syringe possibly weakening the bond between the barrel and the insert. The syringes must not be soaked in any solvent for the same reason.

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