Operating Instructions

For

LiposoFast® LF-50

Figure 1: LiposoFast-50

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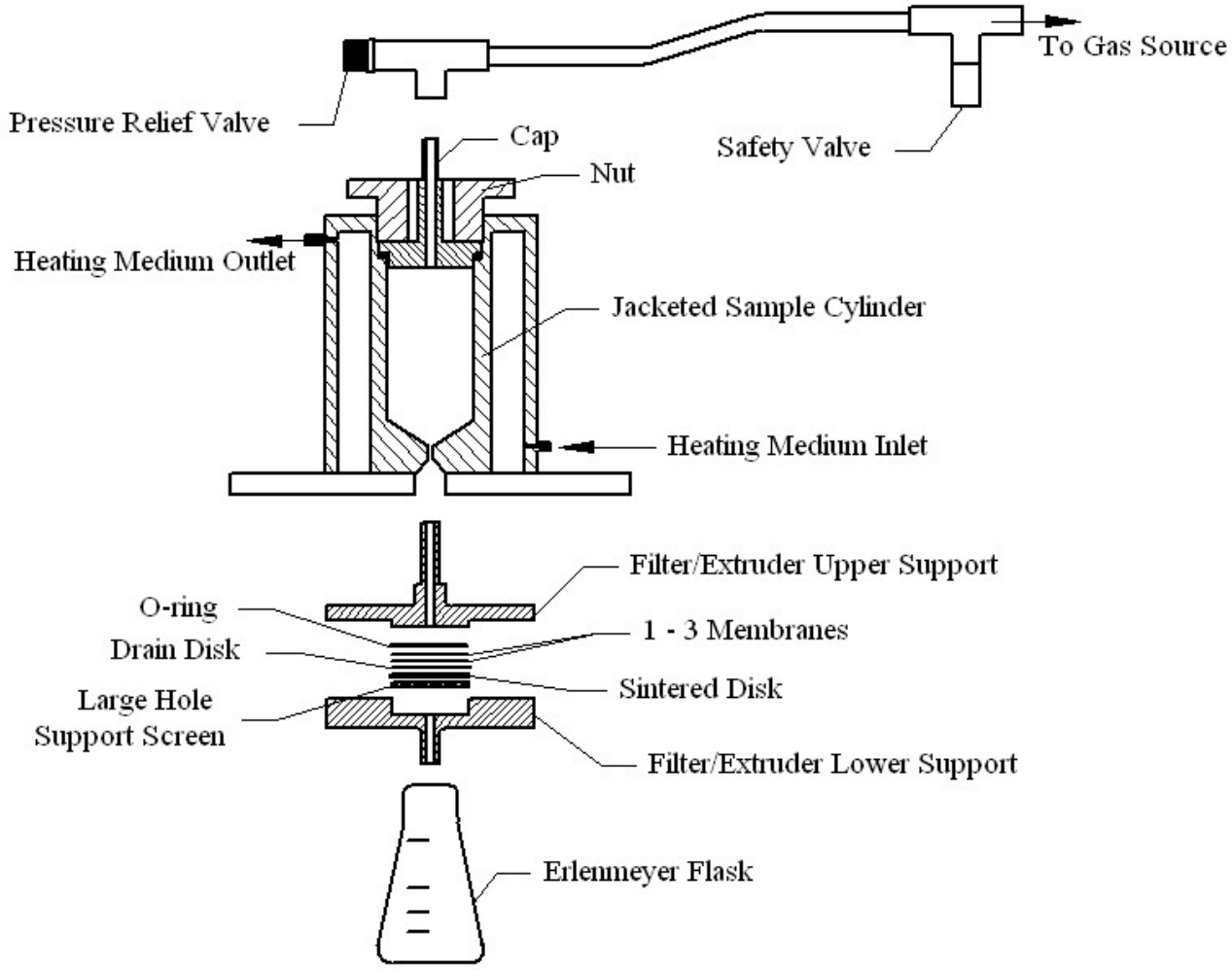

Figure 2: Schematic representation of LF-50 Extruder

1 WARNINGS and General Information:

1.1 Safe Operation:

1.1.1 Incorrect operation may lead to discharge of fluids under pressure. Users must wear eye protection at all times. Gloves and protective clothing must be worn and machine must be operated in a contained area when working with pathogenic, toxic, or corrosive materials. All necessary precautions must be taken to ensure safe operation.

1.1.2 The ¼" flexible Stainless Steel hose has a pressure release valve fitted to the end connected to the sample cylinder. Open the release valve to relieve gas pressure in the line and sample cylinder before opening the sample cylinder. The other end of this hose is designed to be connected to a cylinder of compressed gas. This connector is fitted with a safety valve preset to release at 600psi/41bar. Pressure exceeding 600psi/41bar will cause this safety valve to release accompanied by a very loud noise. This release is not harmful. The valve will reset itself when you reduce the gas pressure below 600psi/41bar.

1.1.3 Do not run the LiposoFast LF-50 without first reading the Operating Instructions (Section 2). Injury to personnel or damage could result

from incorrect operation.

1.1.4 To become familiar with the proper operation of the LF-50, practice using distilled water.

1.2 Operating Principles:
The LiposoFast LF-50 is a medium pressure extruder which uses compressed gas at pressures up to 600psi/41bar to pressurize the sample cylinder and force the starting materials through a polycarbonate membrane. This process is used to reduce the particle size of emulsions and liposome preparations. The processed sample is collected in a 50mL flask.

1.3 Temperature Control:
The central sample cylinder of the LiposoFast LF-50 is sealed within a stainless steel jacket. Heat transfer fluid, usually headed water, is circulated between the sample cylinder and external jacket. The temperature of the starting material can be increased or decreased by changing the temperature of the circulating water. Low jacket volume and high flow-through allow optimum heat transfer.

1.4 Capacity:
The LiposoFast LF-50 has a maximum batch size of 50mL. Samples as small as 5mL can be processed. If the polycarbonate membrane has been pre-wetted almost all of the sample can be recovered. Larger capacity instruments are also available.

1.5 Specifications:
The LiposoFast LF-50 is constructed of corrosion resistant materials. Sample temperatures may be as high as 200ºC. All fittings, hoses and tools are included. The diameter of the extrusion membrane is 25mm.

1.6 Requirements for Operation:
A pressurized gas cylinder and a heating medium (usually warm water) are required.

2 Instructions for Use:

2.1 Preparing multilamellar liposomes (MLV): Dissolve phospholipids in organic solvent and then remove solvent in a rotary evaporator to produce a lipid film. Hydrate the film by adding aqueous phase and shaking by hand or mechanical shaker. This is only one of several common methods to produce MLV's.

2.2 Wipe down device with ethanol prior to use.

2.3 Assemble the filter/extruder as shown in the diagram. Position the large holed support screen followed by the sintered disk into the base of the filter/extruder. Next, place a polyester drain disk onto the sintered disk. Place one to three polycarbonate filter membranes onto the drain disk. The membranes are shiny, opaque circular sheets lying between two protective, blue, paper layers. Position the o-ring on top of the membranes and secure the top of the filter/extruder to the base by tightening the four brown screws provided.

2.4 Attach entire filter/extruder unit to the bottom of the sample and tighten gland gently with wrench.

2.5 Close release valve (clockwise) gently with wrench. Connect stainless steel hose to gas source gently using wrench.

2.6 Unscrew cap of sample cylinder. Add multilamellar lipid vesicle (MLV) sample (max. 50mL per batch). Firmly screw cap shut.

2.7 Turn gas supply on. Using a regulator on the tank, increase pressure gradually until homogenate appears. Applied pressure need not exceed 600psi/41bar. The safety valve on the air hose has been set to release at 600psi/41bar. This release is not harmful but is accompanied by a very loud noise. Reduce pressure to reset safety valve. Required pressure is primarily dependent on the lipid concentration in the MLV

emulsion
and on the number of polycarbonate membranes used (one is the most common). Excessive pressure can cause polycarbonate membranes to rupture. Collect the processed sample in the Erlenmeyer flask.

2.8 Gas supply should be turned off before all of the starting material has been processed. After turning off the gas, prepare to open the release valve as soon as the product has been completely extruded. The use of the release valve prevents gas expulsion into the processed sample which may cause foaming or splashing of the material.

3 Instructions for Temperature Control:

3.1 Connect the hoses provided to the inlet and outlet of jacket. Inlet is the lower connector.

3.2 Attach the inlet tube to the source of the heat transfer fluid. Make sure the outlet tube goes to an appropriate location for collection or draining of the heat transfer fluid.

3.3 Start the flow of heat transfer fluid.

3.3 Adjust flow rate and temperature of heat transfer fluid until desired starting material temperature is reached.

3.4 Proceed with processing as described above.

12 Scaling Up, Scaling Down

12.1 Scaling Up:
AVESTIN manufactures standard and custom made EmulsiFlex homogenizers and filter/extruders with sample sizes from as small as 7mL up to 1000L/h continuous flow at pressures up to 30000psi/207MPa. Contact AVESTIN, or their agent, concerning larger homogenizing equipment.

12.2 Scaling Down:
The LiposoFast handheld extruder is available for the preparation of liposomes/emulsions in small volumes from 0.1mL. Extrusion is through polycarbonate membranes of defined pore sizes. Please contact AVESTIN, or their agent, for more information on the LiposoFast extruders.

Figure 3: Standard EmulsiFlex homogenizers. Call AVESTIN for details.