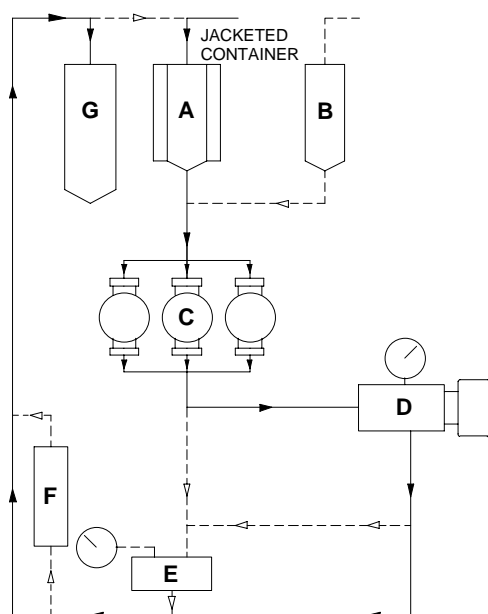


# Operating Instructions for the EmulsiFlex<sup>♦</sup>-C5 High Pressure Homogenizer



## EmulsiFlex Principle of Operation:

A high pressure pump (C) pushes the product through an adjustable homogenizing valve (D). The product can also be passed through a membrane (E). It can be collected (G) or recycled to the reservoir (A) via tubing/pipes or heat exchanger (F).

EmulsiFlex equipment can be delivered as:

- 1-High pressure homogenizer (A,C,D,F,G)
- 2-High pressure filter/extruder (A,C,E,G)
- 3-Homogenizer/filter/extruder combination (A,C,D,E,G)
- 4-Two-phase homogenizer/filter/extruder with precision metering pumps, or as shown to the left (A,B,C,D,E,F,G).

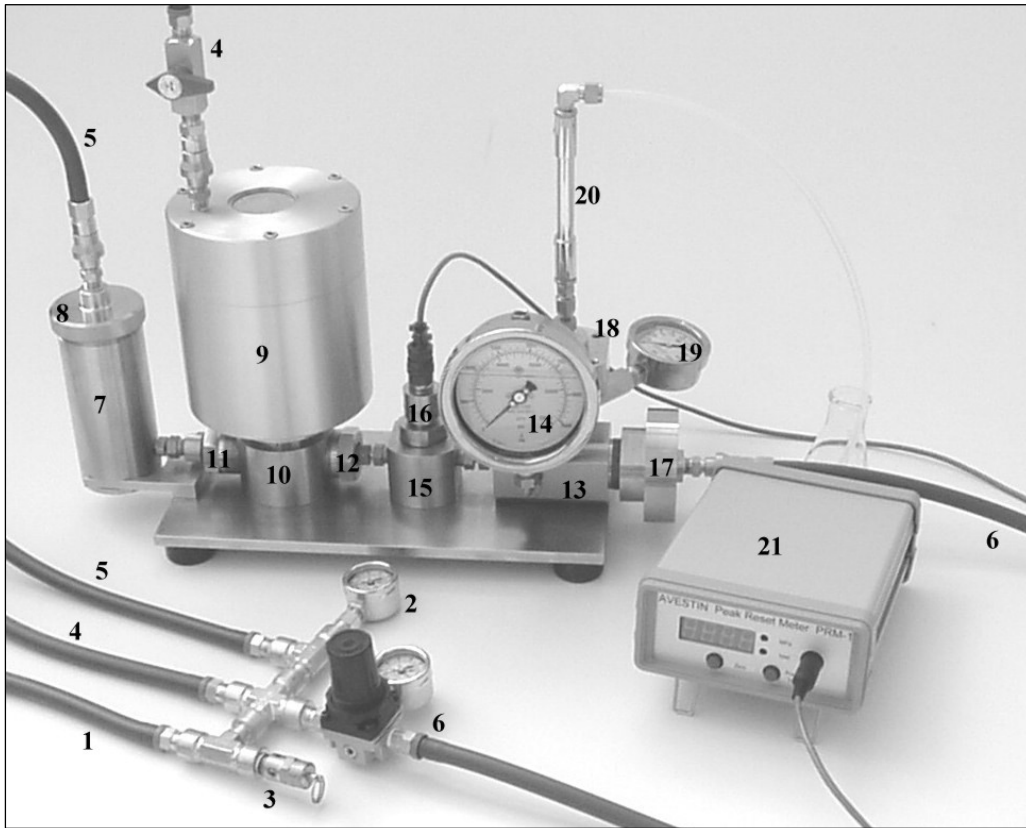
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No voice mail: a qualified person will answer your call in the language of your choice: English, Deutsch, Français



**Figure 1:**

- |    |  |    |  |
|----|--|----|--|
| 1  | hose to connect to air/gas supply                | 12 | outlet check valve                               |
| 2  | gauge to measure incoming air/gas pressure       | 13 | homogenizing valve                               |
| 3  | safety valve                                     | 14 | homogenizing pressure gauge                      |
| 4  | hose with control valve for pump motor           | 15 | in-line transducer adapter (optional)            |
| 5  | hose/Quick Connect to pressurize sample cylinder | 16 | pressure transducer (optional)                   |
| 6  | regulator/hose assembly for pneumatic control    | 17 | pneumatic control cylinder                       |
| 7  | sample cylinder body                             | 18 | filter extruder assembly (optional)              |
| 8  | sample cylinder cap                              | 19 | extrusion pressure gauge (optional)              |
| 9  | pump motor                                       | 20 | heat exchanger (optional)                        |
| 10 | pump body  | 21 | peak reset meter with digital display (optional) |
| 11 | inlet check valve                                |    |  |

## **1. WARNINGS and General Information:**

**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 instrument 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.

**1.2** Do not run the instrument dry for more than a few cycles. If the instrument is not lubricated by a liquid, the

plunger can seize in the pump. This will significantly reduce the lifetime of the seal in the pump.

**1.3** Only run clean fluids through the instrument. Contaminating particulate matter can block the check valves and prevent the instrument from operating properly.

**1.4** Your instrument can have minor physical differences to the picture on the front of this manual. This is due to improvements or technical advancements. The changes do not alter any of the functions described below.

**1.5** The regulator (6) is not a high pressure regulator; the hose assembly should not be attached to incoming gas with a pressure higher than 145psi/1.0MPa. This has to be taken into consideration when using bottled gas which can have a pressure of 3000psi/20.7MPa or more.

**1.6** If the inlet air/gas pressure exceeds 145psi/1.0MPa the safety valve (3) will release the excess pressure with a loud noise. If this happens, reduce the pressure from the compressed air/gas supply immediately. The release of pressure from the safety valve is harmless but very loud.

**1.7** Use cold tap water to learn how to operate the instrument. Experiment with various pressures and flow rates. The minimum sample volume is 7mL and the flow rate is 1-5L/h depending on the pressure. Do not attempt to run toxic, corrosive, or small sample volumes (less than 20mL) until you are familiar with the instrument.

**1.8** The EmulsiFlex-C5 homogenizer has a air/gas-driven high-pressure pump developed by AVESTIN. There is no single "O" ring in the entire path of the product. The only plastic seal in the pump body is the UHMWPE (Ultra High Molecular Weight Polyethylene) plunger seal. There is a PTFE (Teflon) seal for the homogenizing valve stem. The intensifying factor of the pump motor is 240:1. This means that the pressure on the product can go up to 240 times the incoming air/gas pressure which is the only energy source needed to run the pump.

**1.9** Do not use excessive force when tightening any nuts in this instrument. Only tighten enough to prevent leakage. Contact AVESTIN (avestin.com) directly if you have questions; please have your serial number ready.

**1.10** Please retain the wood shipping container in which your EmulsiFlex- C5 was delivered. This container should be used if you need to return the instrument to AVESTIN for updating or servicing (see Figure 20).

**1.11** Do not attempt to disassemble the inside of the homogenizing valve (13) without first consulting with AVESTIN. Please have the serial number of your instrument available when you contact us.

**1.12** EmulsiFlex homogenizers do not have a name plate anywhere. They are a source of dirt and are almost impossible to clean underneath. Information is engraved onto the base plate.

**1.13** The discharge tubing is connected to the hose barb (22 in Figure 4) by means of friction. The hose barb must be clean and dry to ensure a firm grip of the tubing.

Otherwise the tubing will disconnect suddenly and the product will spray around.

**1.14** Avoid scratching any sealing or internal surfaces. Since there are no "O" ring seals in the pump body and homogenizing valve, parts must be precision machined and hardened. This makes it possible to disassemble and reassemble the check valves and homogenizing valve repeatedly without need for any spare gaskets. This is a unique and unmatched feature of EmulsiFlex homogenizers.

**1.15** The homogenizing valve of the EmulsiFlex can be stalled. After the pump is primed and is pumping fluid, stalling occurs when the pump motor is brought to a near halt by closing the homogenizing valve entirely.

**1.16** Periodically check to ensure that the pump motor (9) is securely screwed into the pump body. Do this by turning the pump motor clockwise until it is snug.

## **2. Requirements for operation:**

### **2.1 Compressed air/gas:**

The EmulsiFlex-C5 unit requires a compressed air or gas source. Any non explosive and non-dangerous gas, such as nitrogen, can be used. The required pressure is 240 times less than the desired hydraulic pressure on the product plus 10%. For example: if the product has to be run at 14500psi/100MPa the air/gas pressure will have to be equal or higher than  $14500/240+10\%=66\text{psi}$  ( $100/240+10\%=0.46\text{MPa}$ ). If the incoming pressure exceeds 145psi/1.0MPa, the safety valve will release the pressure with a loud but harmless noise. Reduce the pressure immediately. The maximum possible pressure on the product is 30000psi/207MPa despite the fact that on some EmulsiFlex the maximum is given as 25000psi/172MPa. For most applications an incoming pressure of 80-100psi/0.55-0.69MPa is sufficient. A 3HP/2.3kW compressor (or stronger) is sufficient.

### **2.2 Cooling fluid:**

If a heat exchanger is used there are two ways of supply the cooling medium: A- connect the heat exchanger tubing to a cold water tap. B- use a small pump (e.g. peristaltic pump) to supply the heat exchanger with cooling medium such as ice water.

If a cooling coil is used, a basin of ice water is required to immerse the coil in. Additional ice should be added as needed.

### 2.3 Electric power:

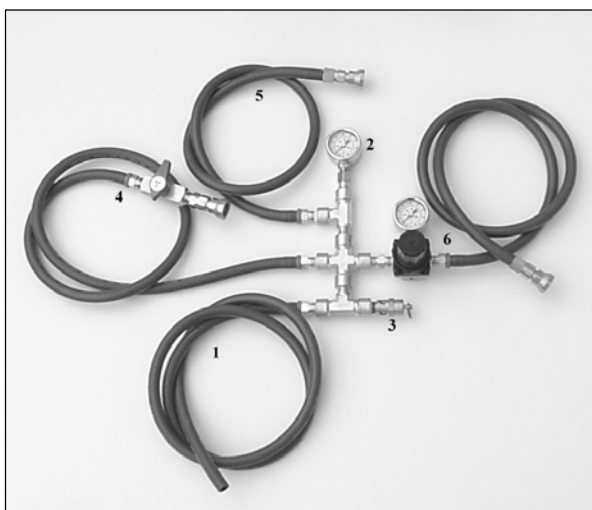
If the optional peak-reset meter (21) is used, it is run on 115V or 220V electrical power at either 50 or 60Hz.

## 3. Operating instructions:

### 3.1 How to run the pump:

**3.1.1** Gas cylinders are usually under pressures up to 3000psi/20.7MPa or more. If you are using a gas cylinder, make sure a regulator (not supplied) is installed on the gas cylinder and set the pressure at or below 145psi/1.0MPa. If you are using compressed air coming from a house compressor there is usually no need for a separate regulator since the pressure of the air coming from a compressor does not usually exceed 145psi/1.0MPa.

**3.1.2** Connect the compressed air/gas supply hose (1) to the air compressor or gas cylinder. The small gauge (2) on the hose assembly reads the pressure which will reach the pump motor. See Figure 2.



**Figure 2:** Close-up of hose assembly showing pressure gauges, safety valve and homogenizing pressure regulator.

**3.1.3** Make sure the control valve (4) is in the horizontal position shown in Figure 3.

**3.1.4** Position the Quick Connect fitting on the end of the hose attached to the control valve (4) at the top of the pump motor (9). Push on the fitting until it snaps on (see Figure 3).



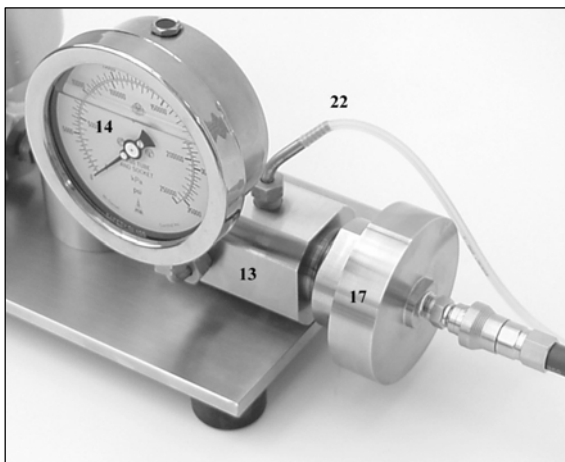
**Figure 3:** The Quick Connect fitting on the control valve (4) is attached to the pump motor. Note that the control valve is in the horizontal position; the pump motor is off.

**3.1.5** Put your sample into the sample cylinder body (7). Do not close the cap (8). This cylinder has a capacity of 250mL. Although the instrument can run samples as small as 7mL, your first tests should be done with the sample cylinder at least half-full of cold tap water. Place the free end of the discharge tubing into an empty flask or attach the tubing to the side of the sample cylinder to recycle the water, as shown in Figure 3.

**3.1.6** Start the pump motor by turning the control valve knob (4) to the vertical position. As the pump starts to cycle the pump plunger will move up and down quickly inside the pump. The sample will be sucked through the inlet check valve (11) into the pump body and pumped through the outlet check valve (12) and into the homogenizing valve (13). The homogenizing pressure shown on the gauge (14) or optional electronic meter (21) will be low because the homogenizing valve is open. The pump motor can be stopped at any time by turning the green control valve (4) back to the horizontal position. Let the pump run for approx. 60 seconds. The sample should now be pumping into your flask via the outlet tubing. If no sample passes through the instrument, close the cap of the sample cylinder and connect the second Quick Connect (5) to pressurize the sample. After the pump is primed the cap can be removed after first removing the Quick Connect from the cap. If the sample is viscous (e.g. yoghurt), the sample cylinder must be kept under pressure to help the pumping process.

**3.1.7** Adjust the homogenizing pressure by adjusting the pressure in the pneumatic control cylinder (17). This is done by changing the pressure setting at the pressure regulator (6). The pressure read on the big gauge can be adjusted from less than 500PSI/3.4MPa up to maximum of 240 times the pressure read on the small gauge (2). Turning the regulator knob clockwise increases air pressure in the cylinder, closes the gap, reduces the flow rate and increases the homogenizing pressure. Turning counter-clockwise decreases air pressure in the control cylinder, opens the gap, increases the flow rate and reduces homogenizing pressure.

Do not connect the regulator/hose assembly (6) to the pneumatic control cylinder (17) with pressure in the hose. Make sure the regulator gauge reads zero. Increase pressure as required after connection is made.



**Figure 4:** Close up of the pneumatic pressure control cylinder (17) attached to the homogenizing valve (13). Also shown is the sample outlet tubing attached to a hose barb (22).

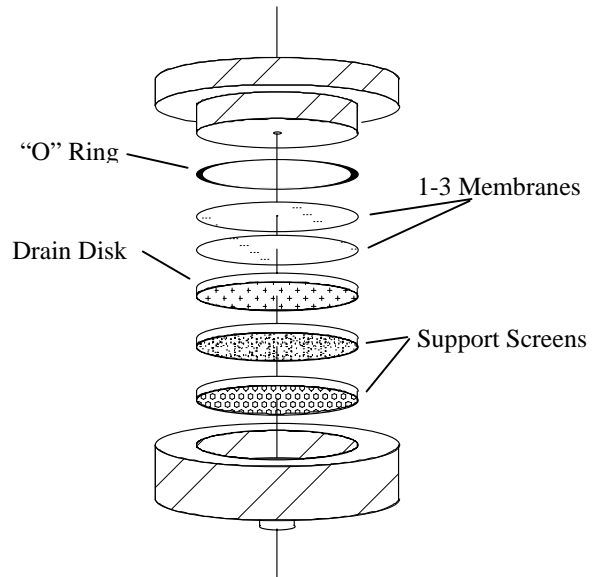
**3.1.8** As soon as the sample cylinder is empty the pump motor will speed up. Turn the green knob (4) to the horizontal position to stop the pump.

**3.1.9** The compressed air/gas that drives the pump motor does not come in contact with samples during processing. The sample cylinder can also be connected separately to an inert gas supply with pressure less than 145psi/1.0MPa. When processing volumes greater than 1L it is less costly to run the EmulsiFlex-C5 with an air compressor than with bottled gas.

## 3.2 How to operate the filter/extruder:

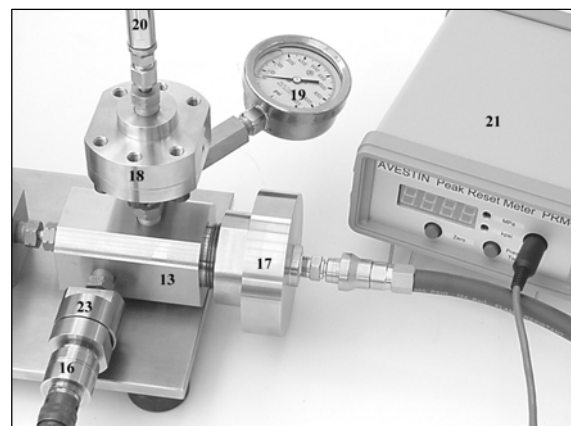
**3.2.1** Assemble the filter/extruder system as shown in Figure 5. Use the brown stainless steel screws provided to

secure the filter/extruder unit. Only the provided brown screws should be used. The brown colour is the result of the hardening process giving them the extreme strength required. Tighten screws with the slot key provided. These screws have to be tightened firmly to make sure they do not loosen and open during processing.



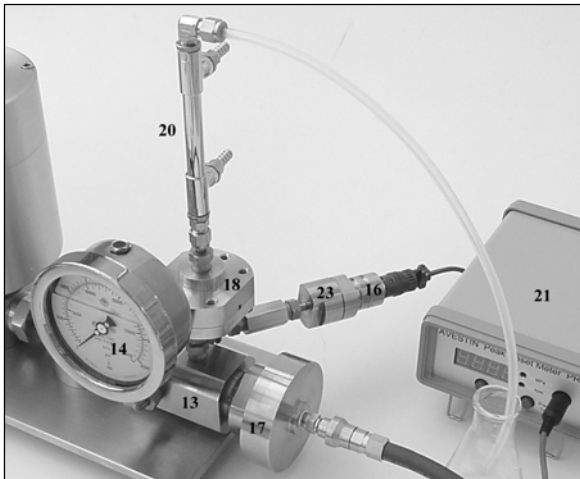
**Figure 5:** Filter/extruder assembly.

**3.2.2** Mount the filter/extruder as shown in Figures 6 and 7 using the wrench provided. Do not over-tighten the gland nuts.



**Figure 6:** Homogenizing pressure can be measured using a pressure transducer (16) attached to an electronic meter (21). Filtration/extrusion pressure can be measured using a pressure gauge (19) or with a pressure transducer and electronic meter (see Figure 7). Note the optional transducer adapter (23).

**3.2.3** The pressure of the filtration/extrusion process can be monitored by a gauge (19) as shown in Figure 6. This gauge is supplied with all filter/extruder units. If using the optional peak-reset meter, the pressure transducer (16) must be mounted as shown in Figure 7. The maximum pressure allowed is approximately 6500psi/44.8MPa. Be careful not to exceed this pressure. Viscous or other unsuitable products will not extrude and the pressure will exceed the allowable level. Product will be released through the safety venting grooves in the extruder.



**Figure 7:** Heat exchanger (20) mounted onto the filter/extruder (18). An outlet tube is fitted to the heat exchanger. Tap water or heat transfer fluid is passed through lines attached to connectors on the sides of the heat exchanger. Note that a pressure transducer (16) can be attached to measure the filtration/extrusion pressure using an optional transducer adapter (23).

**3.2.4** Samples can be processed through the filter/extruder with or without homogenizing. If the homogenizing valve is open, the sample will be filtered/extruded only. In this case, the homogenizing pressure gauge (14) shows the same pressure as the extrusion pressure gauge (19). Closing the homogenizing gap by increasing pressure in the regulator/hose assembly (6) will increase the pressure on the gauge (14). In this case, the product is homogenized and the pressure measured by the homogenizing pressure gauge (14) and extruded at the pressure measured by the extrusion pressure gauge (19). This is a unique process.

### 3.3 Cooling the sample:

**3.3.1** Processing samples repeatedly and/or at high pressures causes the product to heat up. For temperature sensitive products, a heat exchanger (20) is available from AVESTIN. The heat exchanger can be installed without

any modification to the EmulsiFlex as shown in Figure 7. AVESTIN also supplies simple cooling coils where less stringent cooling is required (see Figure 8). The EmulsiFlex can also be stored and operated in a cold room to minimize product temperature increases.

### 3.4 Small sample volumes:

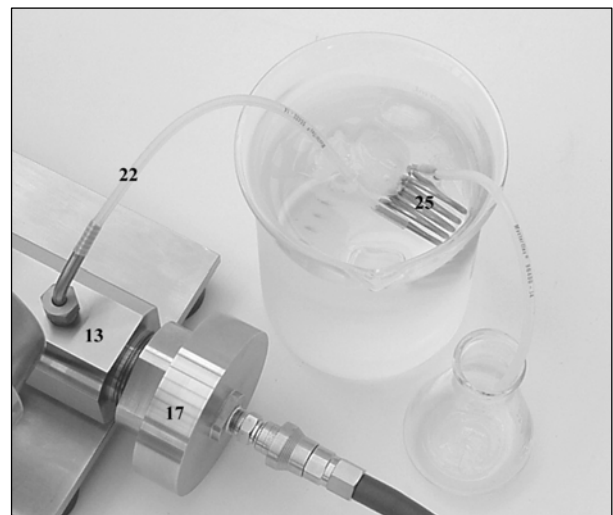
The minimum possible sample volume for the EmulsiFlex-C5 homogenizer is 7mL. When combined with the filter/extruder the minimum volume is 10mL. To run a small sample proceed as follows:

**3.4.1** close the homogenizing valve entirely by fully pressurizing the pneumatic control cylinder (17).

**3.4.2** put your sample into the sample cylinder and close the cap

**3.4.3** connect the hose (5) to the sample cylinder cap. Now the sample is under pressure in the sample cylinder.

**3.4.4** start the pump motor and adjust the pressure on the homogenizing valve. You can either: A- collect the sample and repeat the process if necessary. B- stop the pump when it is primed (as soon as product comes through the outlet tubing). Open the sample cylinder cap. Insert the outlet tubing into the sample cylinder as in Figure 3. Start the pump again and run the small sample in a closed cycle.



**Fig. 8:** Cooling coil (25) attached to homogenizing valve outlet, in beaker of ice water.

### 3.5 Viscous samples:

Put your viscous sample into the sample cylinder (7) and close the cap (8). Connect the compressed air/gas supply

hose (5) to the Quick Connect fitting on the sample cylinder cap. Start the pump and control the homogenizing pressure with the regulator (6). Samples as viscous as mayonnaise or highly concentrated liposome formulations have been processed successfully. This should be satisfactory for most of the applications. Extremely viscous samples will result in air pockets forming in the sample reservoir. To avoid this, a piston for the sample cylinder is available to place on top of the sample to help push the product through the inlet check valve of the pump. This special tool is not standard equipment, but can be purchased from AVESTIN under catalogue number C5CYLPLU1 (shown in Figure 9).



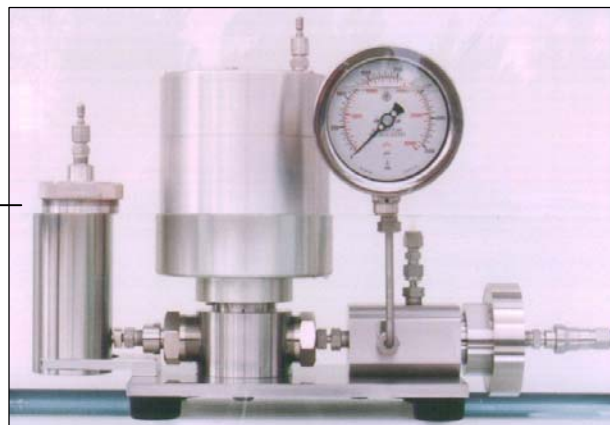
**Figure 9:** Sample cylinder piston (24) is used to help push viscous samples into the pump.

### 3.6 High temperature applications:

The entire product path of the EmulsiFlex-C5, including the sample cylinder and the filter/extruder assembly can be immersed in a temperature controlled water bath. Temperatures up to 160°F/70°C are possible. Do not submerge the pump above the water line as shown in Figure 10.

### 3.7 Processing samples for more than one pass:

**3.7.1** Samples can be processed in continuous passes. After the pump has been primed, turn the pump off by closing the pump motor control valve (4). Remove the sample cylinder cap (8) and introduce the flexible outlet tubing into the sample cylinder body (Figure 3). Secure the tubing to prevent accidental spraying of product. Turn the pump on and run the sample in a closed cycle. To determine the number of passes required, measure the flow rate and the process time. This procedure is approximate only. For a more precise count of the number of passes, the following procedure is recommended:



**Figure 10:** The EmulsiFlex-C5 can be submerged in a temperature controlled water bath to the level shown

**3.7.2** Let the entire sample pass through the instrument, then return it to the sample cylinder and repeat. This ensures homogenization and/or filtration/extrusion of the entire sample every cycle and allows the number of cycles to be monitored.

### 3.8 Large sample volumes:

After priming, and while fluid is being pumped, the sample cylinder cap (8) can be removed from the top of the sample cylinder in order to add more sample or to recycle the product. Do not remove cap if the sample is viscous, as continuous pressure in the sample cylinder may be necessary to push the sample into the pump. The EmulsiFlex-C5 is not designed for production size volumes. It is a basic, versatile laboratory instrument. AVESTIN also manufactures larger, pilot plant and production volume equipment (Figure 18).

### 3.9 How to operate the peak-reset meter:

Figure 6 shows the peak-reset meter (21) and pressure transducer (16) set up to read the homogenizing pressure. A peak-reset meter can also be used to measure the extrusion pressure (Figure 7). Peak-reset meters can be run at 115V or 220V at 50 or 60Hz with pressure readings in kpsi or MPa. After connecting the peak-reset meter to a power supply, connect the pressure transducer to the meter with the supplied cord. Make sure the transducer is installed in a transducer adapter and attached to the homogenizer. Switch on the power at the back. The meter displays the peak pressure reading approximately every second. Pressure measurements can be downloaded into a computer via an RS232 port at the back of the meter. Contact AVESTIN for details. More detailed instructions are found in the peak-reset meter manual which comes with every unit.

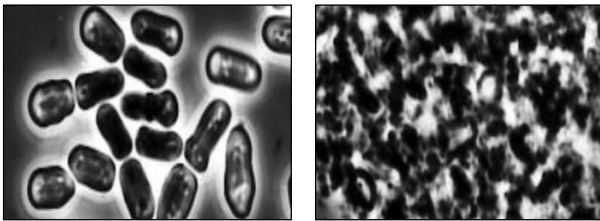


#### 4. Specific Applications:

The EmulsiFlex-C5 is capable of handling an extremely wide range of applications. Only a few of the most common applications are outlined below. In almost every case it will be necessary for the individual operator to work out details specific to their own applications.

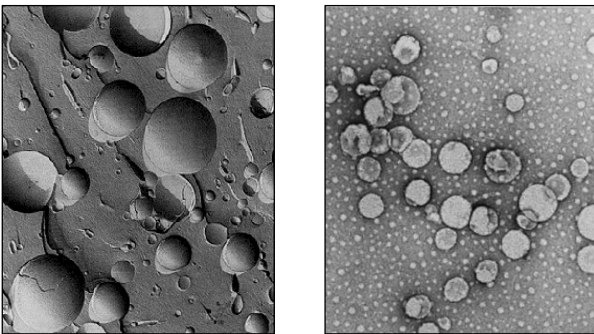
##### 4.1 Cell rupture:

One pass through the homogenizer at 15000-17000psi/103-117MPa has been shown to rupture almost all of the cells in a batch of *E. coli*. More resistant cells, including *Piccia*, *Saccharomyces* and *Mycobacterium*, can be ruptured at higher pressures (>22000psi/152MPa) with multiple passes.



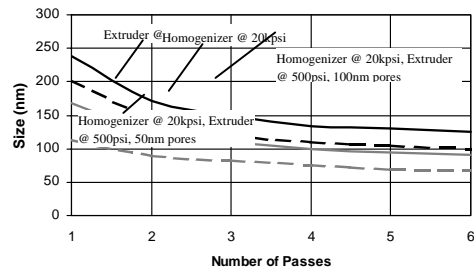
**Fig. 11:** Cell rupture by an EmulsiFlex high pressure homogenizer. Undisrupted *Schizosaccharomyces pombe* (yeast) cells (left) and cell fragments after processing at 28000psi/193MPa.

##### 4.2 Liposomes:



Exact parameters will depend on the components and their concentration in each formulation. In general, 4 passes through the homogenizer at 15000psi/103MPa are sufficient to form a homogeneous population of unilamellar liposomes. Liposome size can be controlled by passing the sample through the filter/extruder holding a polycarbonate filter. Homogenizing, followed by extrusion, reduces the number of passes required to form liposomes of a defined size.

**Fig. 12:** Liposomes prepared using 100nm membranes.



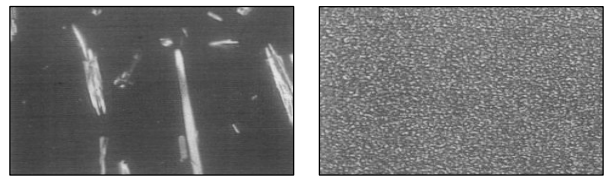
**Fig. 13:** Preparation of liposomes by extrusion alone, homogenizing, and combined homogenizing/extrusion.

##### 4.3 Emulsions:

Exact parameters will depend on the emulsion components and their concentrations. In general, 1-4 passes through the homogenizer at 10000-20000psi/69-138MPa are sufficient to form a homogeneous emulsion. If this does not work for your sample, try altering the number of passes through the EmulsiFlex or different homogenizing pressure (either higher or lower). Two or more sample reservoirs can be installed with micrometer adjustments. With this design the emulsion components can be mixed just prior to homogenizing. Contact AVESTIN directly regarding this arrangement.

##### 4.4 Dispersions:

The size of dispersion particles can be dramatically reduced by processing through the EmulsiFlex-C5 through its entire pressure range. If the starting material is not stable (particles separate out quickly), it is often useful to run a first pass at low pressure (approx. 5000psi/35MPa) to break down the largest particles. Pressure can be increased incrementally from this point



for difficult to process materials.

**Fig. 14:** Unprocessed dispersion (10-100microns, left) and processed, uniform dispersion (1micron, right) using an EmulsiFlex-C5 at 25000psi/173MPa.

#### 5. Protocol to Clean/Sterilize the EmulsiFlex-C5:

##### 5.1 Cleaning:

**5.1.1** After the sample has been processed and the pump is no longer pumping, switch off the pump motor. A small



residual sample will remain in the pump body and the nipple connecting the pump outlet check valve to the homogenizing valve. Leave the homogenizing valve open and flush out the residual sample by pressurizing the sample cylinder.

**5.1.2** Open the nut connecting the sample cylinder to the inlet check valve as shown in Figure 15. Slide off the sample cylinder and clean it with an appropriate cleaning agent which will depend on the sample processed. The sample cylinder is made of type 316L stainless steel, the most corrosion resistant steel. Almost any type of cleaning agent can be used.

**5.1.3** Put the sample cylinder back in place and tighten the nut gently. Fill the sample cylinder with the appropriate cleaning agent and pump it through the EmulsiFlex with the homogenizing valve open. You will know that the homogenizing valve is fully open if there is no pressure reading on the large gauge (14). Do not use organic solvents such as chloroform, ethyl/diethyl ether, phenol, or petroleum based compounds for cleaning. These can damage the pump seal. Alternative seals can be used if necessary. Common cleaning agents include soapy water, ethanol and acetone.



**Figure 15:** Open the nut connecting the sample cylinder (7) to the inlet check valve (11) using the wrench provided.

**5.1.4** After the cleaning agent is through, flush the residual volume through the instrument as explained in 5.1.1.

**5.1.5** If there is a high degree of cleanliness required, the following steps can be taken in addition to the points above:

**5.1.5.1** Prior to and following cleaning, remove the nipple connecting the gauge to the homogenizing valve and clean thoroughly.

**5.1.5.2** The gauge inlet can be cleaned by using a syringe to flush out the extremely small amount of product which might be trapped.

**5.1.5.3** The check valves and homogenizing valve can be disassembled for cleaning and inspection. This has to be done by a technically knowledgeable person following the EmulsiFlex check valve and homogenizing valve manual. Contact AVESTIN directly if you do not have this manual; please have your serial number available.

## **5.2 Steam-in-place sterilization:**

**5.2.1** Clean the EmulsiFlex as explained in 5.1. Step 5.1.5.1 is recommended. Leave the homogenizing valve open.

**5.2.2** Close the sample cylinder cap and connect a steam source to it. Run steam through the EmulsiFlex at temperatures not exceeding 255°F/125°C. Steaming duration depends on the nature of the application.

**5.2.3** Allow the EmulsiFlex to cool down below 160°F/70°C before operating.

**5.2.4** Do not use the blue hoses supplied with the standard EmulsiFlex. AVESTIN offers a flexible stainless steel hose for steam sterilization. The tubing and fittings can also be purchased at a local Swagelok fitting supplier. Ask AVESTIN for the Swagelok catalogue # for the necessary parts.

## **5.3 Autoclave sterilization:**

**5.3.1** Clean the EmulsiFlex as explained in 5.1. Step 5.1.5.1 is also recommended. Leave the homogenizing valve open.

**5.3.2** Loosen the nut on top of the large gauge (14) to allow the air inside to expand. Just loosen it; there is no need to remove it.

**5.3.3** Remove the blue air/gas hoses from the C5.

**5.3.4** The entire EmulsiFlex can now be put into an autoclave to be sterilized at temperatures below 255°F/125°C for one hour, or according to your standard practices.

**5.3.5** Allow the EmulsiFlex to cool down below 160°F/70°C before operating.

## 6. Troubleshooting:

**6.1** The pump should stall up to 30000psi/207MPa when the homogenizing valve (13) is completely closed (pump and flow of material should slow to a near halt). When this happens the pressure read on the large gauge (14) will be approx. 240 that of the pressure read on the small air/gas gauge (2). If the pump does not stall there are two possibilities:

**6.1.1** The homogenizing valve seat and stem are worn and have to be replaced. Contact AVESTIN to order the replacement parts and an instruction manual for opening the homogenizing valve; please have the serial number available.

**6.1.2** The check valves are not closing properly. See 6.3.2. Pay attention to the inlet check valve (11) first, particularly if the homogenizer is running rough dispersions or fibrous material.

**6.2** Product comes out of the hole on the top of the homogenizing valve (13) near the homogenizing pressure control cylinder (17):

**6.2.1** The Teflon seal is not sealing properly. The pneumatic control cylinder (17) has to be tightened. Use a 2" wrench and tighten the cylinder using the wrench flats provided.

**6.2.2** If the leaking persists after tightening this cylinder, the Teflon seal of the homogenizing valve stem is damaged and has to be replaced. Contact AVESTIN to order a replacement Teflon seal and an instruction manual for opening the homogenizing valve.

### 6.3 Pump is not priming:

**6.3.1** First try to pressurize the sample cylinder. In most cases the pump will start pumping. You can then increase the pressure.

**6.3.2** The check valves are not closing properly. The likely cause of this is contaminating particles such as dirt, particularly fibrous material, entering the check valves through the sample cylinder. This prevents the check valves from closing.

**6.3.2.1** Open the homogenizing valve. Pressurize the sample cylinder and blow air/gas through the EmulsiFlex to remove foreign particles from the check valves.

**6.3.2.2** Disassemble the check valves, clean and reassemble. Ask AVESTIN for instructions to perform this procedure; please have your serial number ready.

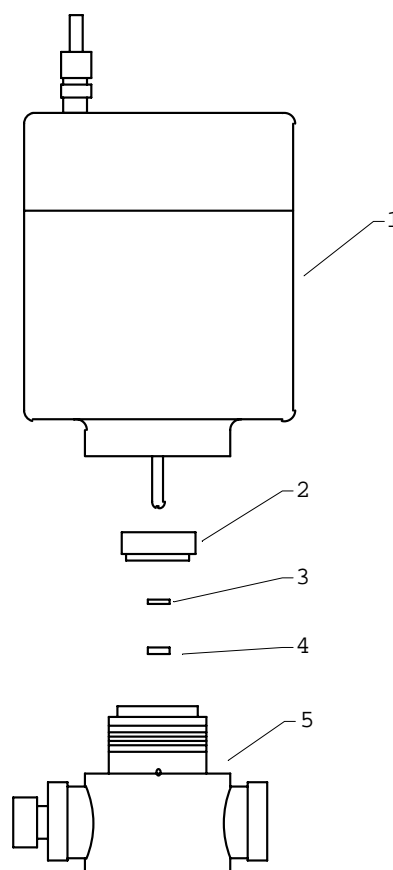
**6.4** Leakage of the product through a relief hole located on the pump body (see Figure 1, 10). Stop using the unit immediately and refer to Section 7 regarding plunger seal replacement/inspection.

**6.5** Bubbles in your processed sample at high pressure come from air/gas incorporated into the product and not from the pump motor. To avoid bubbles use degassed buffer solutions.

## 7. Plunger Seal Replacement:

### 7.1 Seal Removal:

**7.1.1** Place the EmulsiFlex-C5 on a stable, flat surface. Remove the air motor (Figure 16, 1) by turning counter-clockwise. It will slowly unthread itself from the pump body (Figure 16, 5). If the air motor will not turn, put a thick elastic band around the motor for added grip.



**Figure 16:** EmulsiFlex-C5 plunger seal removal.

**7.1.2** Once loose, lift the air motor (Figure 16, 1) **vertically** off of the pump body and set aside. Make sure the motor is secure and does not roll away.

**7.1.3** Lift the round seal back-up support (Figure 16, 2) out of the socket in the top of the pump motor (Figure 16, 5). Set aside.

**7.1.4** The black, seal back-up (Figure 16, 3) is now visible in the top of the pump body (Figure 16, 5). It can be lifted out with care. Set aside or discard; it will not be used again if a new seal set is being installed.

**7.1.5** The white, high pressure plunger seal (Figure 16, 4) is now visible in the top of the pump body (Figure 16, 5). It can be removed, but is fairly tight and must be taken out with care if it is to be used again (in the case of inspection).

**7.1.6** Check the bore down the centre of the pump body (Figure 16, 5) for dirt. Flush with water if not clean.

**7.1.7** If the seal set is just being inspected/cleaned, look at the white, high pressure plunger seal (Figure 16, 4) and black seal back-up (Figure 16, 3) for signs of damage or wear. If any cracks or signs of extrusion are visible, replace both seal parts (Figure 16, 3 & 4). Replacement seals are purchased directly from AVESTIN under catalogue number C5PLSEAL1.

## 7.2 Plunger Seal Insertion:

**7.2.1** It is recommended that the plunger seal insertion tool (Figure 17, 6 & 7) is used for this operation. It can be purchased directly from AVESTIN under Catalogue Number C5SLTOOL1.

**7.2.2** Insert the disk shaped part of the insertion tool (Figure 17, 6) as shown into the top of the pump body. Make sure it is inserted all the way in, and is flat.

**7.2.3** Position the white, high pressure seal (Figure 17, 4) with the **spring down**, in the hole in the centre of the insertion tool (Figure 17, 6). Its orientation can be corrected with the tip of a pen or pencil once dropped in the hole, if necessary.

**7.2.4** Press on the back of the high pressure seal with the insertion plunger (Figure 17, 7) as shown. This compresses the seal slightly as it enters the pump body. Once the seal bottoms out, it is seated correctly.

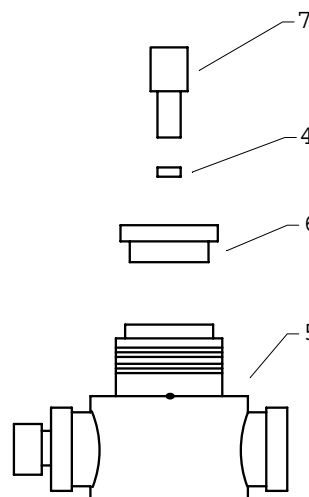
**7.2.5** Remove the disk shaped portion of the seal insertion tool (Figure 17, 6) and inspect the seal. It should be

sitting flat in the bottom of the socket in the pump body (Figure 17, 5). The spring should not be visible as it is face down.

**7.2.6** Take the black, seal back-up (Figure 16, 3) and position it on top of the white, high pressure seal. Push lightly with your finger; it should slide into the socket in the pump body on top of the high pressure seal.

**7.2.7** Replace the steel, seal back-up support (Figure 16, 2). It is inserted as shown and should sit flat in the large socket in the top of the pump body (Figure 16, 5).

**7.2.8** Replace the air motor (Figure 16, 1). It must be inserted vertically, taking great care not to damage the chrome-plated plunger at the bottom of the air motor. Screw motor on clockwise very carefully. If it does not turn easily from the beginning, loosen and start again. It is tight enough when it stops turning. There is no need to tighten the air motor with a tool.



**Figure 17:** EmulsiFlex-C5 plunger seal insertion

**7.2.9** Reattach any hoses/fittings necessary to run the EmulsiFlex-C5. Check the operation of the new seal by running water through the system. Briefly run the homogenizer at high pressure while watching the leak detection hole in the pump body. If no water appears in this hole, the seal replacement is successful.

## 8. Scaling up, scaling down:

### 8.1 Scaling Up:

AVESTIN manufactures a series of standard (Figure 18) and custom made EmulsiFlex homogenizers suitable for volumes as low as a 3mL batch and as high as 2000L/

continuous flow at pressures up to 30000psi/207MPa.

#### 8.1.1 EmulsiFlex-C50:

The EmulsiFlex-C50 is capable of processing at rates between 15 and 50L/hr depending on the homogenizing pressure (up to 30000psi/207MPa). It is powered by compressed air/gas and is electronically controlled for quiet, precise operation.

#### 8.1.2 EmulsiFlex-C160:

The EmulsiFlex-C160 processes at 160L/hr regardless of the homogenizing pressure desired (up to 30000psi/207MPa). It is electrically powered and exhibits very low homogenizing pressure pulsation.



**Figure 18:** Standard EmulsiFlex homogenizers. Custom models are available upon request. Call AVESTIN for details.

### 8.2 Scaling Down:

The LiposoFast line of extruders is ideal for the preparation liposomes/emulsions in volumes from 0.1 to 100mL. Extrusion is through polycarbonate membranes of defined pore sizes.

#### 8.2.1 LiposoFast-Basic:

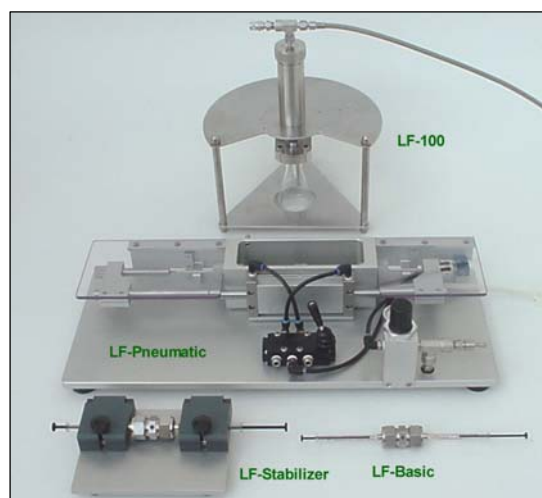
This hand-powered unit is the smallest extruder system made by AVESTIN (0.1mL to 1.0mL). The instrument is made up of two, gas-tight, glass syringes (0.25, 0.5 or 1.0mL) mounted in a housing that holds a polycarbonate membrane. The LiposoFast-Basic has virtually no dead volume and is ideal for preparing a large number of small samples. The LiposoFast Stabilizer has been designed to support the LiposoFast-Basic extruder. This is very useful in the repetitive preparation of highly concentrated emulsions.

#### 8.2.2 LiposoFast Pneumatic

The LiposoFast-Pneumatic uses pneumatic actuators to push a sample (max. 2mL) back and forth through a polycarbonate membrane. Temperature control is provided by an included water bath.

#### 8.2.3 LiposoFast-100 (and –100-Jacketed)

The LiposoFast-100 and LiposoFast-100-Jacketed is for volumes from 2 to 100mL. These medium pressure extruders push liposome suspension/emulsions from a reservoir through a membrane with gas (up to 600psi/4.1MPa). The LiposoFast-100-Jacketed includes a reservoir jacket for fine temperature control.



**Figure 19:** Standard LiposoFast extruders.

### 9. Shipping:

All EmulsiFlex homogenizers are shipped by air in high quality, wood crates. KEEP THIS CRATE as it is the best way to store/transport your homogenizer.



**Figure 20:** EmulsiFlex-C5 on its shipping crate.