Jule Gradient Former
Operator's Manual

Table of Contents

This manual is a guide for the use of the Jule Gradient Former. Data herein has been verified and is believed adequate for the intended use of the product. If the product or procedures are used for purposes over and above the capabilities specified herein, confirmation of their validity and suitability should be obtained, otherwise Jule, Inc. does not guarantee results and assumes no obligation or liability. This publication is not a license to operate under, or a recommendation to infringe upon, any patents.

Jule Gradient Former

The Jule Gradient Former can be used for generating reproducible linear or exponential gradients for electrophoresis, centrifugation, chromatography, and other techniques.

The Jule Gradient Former is supplied assembled and ready for making linear gradients.

Jule, Inc.
185 Research Drive, #6
Milford, CT 06460
www.precastgels.com
Phone: (800) 648-1772
Fax (203) 878-7448

Revised: May 10, 2004

1.0 Theory Of Linear Gradients

To form linear gradients, the Jule Gradient Former is arranged as shown in Fig. 1. A linear gradient is one in which the concentration of the liquid changes at a constant rate with respect to volume.

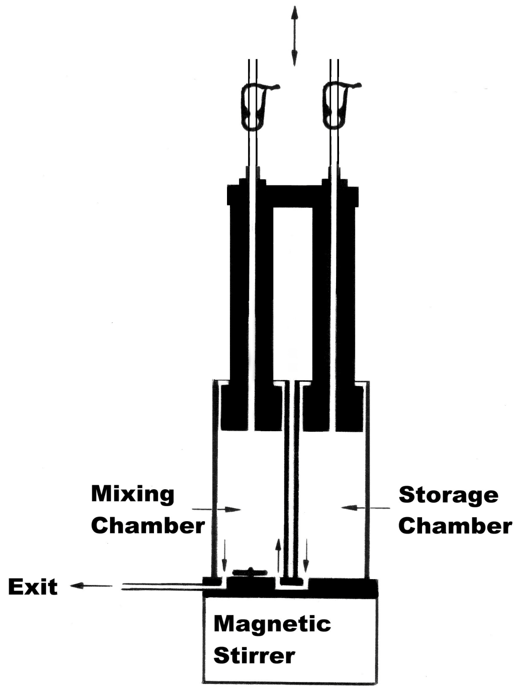

Fig. 1 Linear Gradient Former

To make perfect linear gradients the geometry of the two chambers must be identical, and the chamber fluid levels must drop at the same rate in each chamber during operation. The Jule Gradient Former is an improvement over the basic gradient former because the fluid levels in the mixing chamber and storage chamber are displaced equally. This is accomplished by utilizing the dual piston assembly to dispense the fluid at an equal rate in each chamber, regardless of differences in fluid viscosity or density (see fig. 1.). During operation, liquid flows from the storage chamber into the mixing chamber. The concentration of the stream of gradient exiting from the mixing chamber changes at a constant rate with respect to volume. The result is a perfectly linear gradient varying between the two original limits. The gradient can be increasing or decreasing in concentration, depending upon the relative concentrations initially placed in the two chambers.

The concentration of the stream of the linear gradient emerging from the mixing chamber is given by:

$$Ct = Cm + (Cr - Cm)\ (Vt/2Vo)$$

Where:

Ct = Concentration of gradient being delivered at any time “t”.
Cm = Starting concentration in mixing chamber.
Cr = Starting concentration in storage chamber.
Vt = Volume of gradient withdrawn at time “t”.
Vo = Original volume in each chamber.

Example:

Assume the mixing chamber contains 25 ml of 0% w/v sucrose (plain water) and the storage chamber contains 25 ml of 40% w/v sucrose (i.e., a concentration increasing gradient is being made). At the point in time when a volume of 25 ml has been delivered, the instantaneous concentration of mixing chamber would be:

$$Ct = 0 + (40\text{-}0)\ 25/50$$
$$Ct = 20\%\ \text{w/v sucrose}$$

A few of the numerous linear profiles that can be generated are shown in Fig. 2.

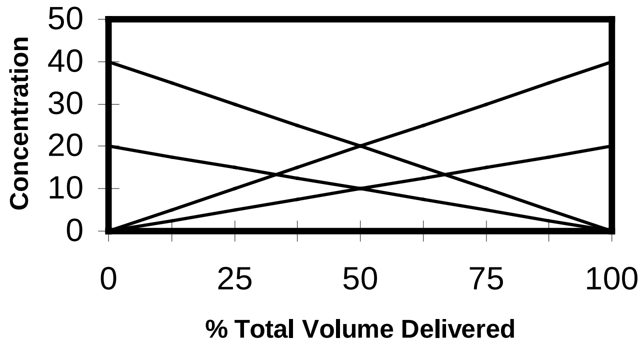

Fig. 2 LINEAR GRADIENT

*Reference "Centrifugation in Biology and Medical Science", Phillip Sheeler.

2.0 OPERATING PROCEDURES FOR LINEAR GRADIENTS FOR MODEL J50
(See Section 2.1 for models J17 and J5)

Note: Make practice gradients before attempting final gradient.
Note: Density gradients can be poured by filling centrifuge tubes or gel cassettes from the bottom up (more dense liquid lifts solution) or from the top (less dense liquid layered on top of higher density liquid). See Top Filling or Bottom Filling below.
Note: Gradient former cylinders are lubricated prior to shipment, but may require lubrication periodically. If cylinders require lubrication, open top two clamps and remove dual piston assembly from cylinders. Using a Q-tip, coat the inner walls of both cylinders with a thin film of high vacuum silicone grease (Dow Corning). Insert the dual piston assembly and move pistons up and down in order to spread the grease along the length of the cylinders.

Manual Filling:
To dispense gradient manually, load the storage and mixing chambers with the appropriate solutions. Close the piston clamps and then open two base clamps. Slowly push on dual piston assembly to allow adequate time for fluid from storage chamber to mix thoroughly in the mixing chamber before exiting. A complete gradient should be poured in 1.5 to 2 minutes.

Automatic Filling:
A peristaltic pump can be attached to the output of the gradient former instead of depressing the pistons manually. Piston assembly will be drawn downward during the gradient forming, thereby keeping fluid levels equal in each chamber.

TO OPERATE LINEAR GRADIENT FORMER

1) Refer to Fig. 1. During Operating Procedures.
2) Open top two clamps and remove dual piston assembly from cylinders.
3) Close off the two flexible tube clamps at the base of the gradient former. One clamp shuts off flow from the storage chamber to mixing chamber and the other shuts off flow from the mixing chamber exit.
4) Place a small Teflon-coated stir bar magnet into the mixing chamber.
   (Example: ½" long x 1/8" diameter for model J50)

5) Volume indicator label should be used for reference only.
6) Place gradient former on magnetic stirrer and turn on mixer to as high a speed as possible to assure thorough mixing.
7) Follow steps below for either top filling or bottom filling.
Note: The stir bar in the mixing chamber prevents pistons from traveling to the very bottom of gradient former. If it is desirable to dispense the small amount of fluid remaining, an air gap should be left between the liquid and pistons. The air will push out the remaining few drops. Tilt gradient former to position last drops of liquid over holes. Stop pistons before air bubbles enter density gradient to prevent unnecessary stirring, if you are "bottom filling" the gradient.
Note: One way to create an air gap is to first fill each chamber to desired volume. Next, insert assembly to within 1/8" or ¼" of liquid. Close piston clamps and air gap is created. Air pushes out the final drops of liquid.

Top Filling :
Gradients can be made by filling from the top by allowing the more dense liquid to enter first and layering less dense liquid on top.

a) Fill the mixing chamber with the desired amount of dense solution for a linearly increasing concentration gradient and pipette the less dense solution into the storage chamber.
b) Follow steps 8 through 12 (see below).

Bottom Filling:
Gradients can be made by filling gel cassette or centrifuge tube from the bottom up by allowing the less dense liquid to enter first and allowing the more dense liquid to raise the less dense to the top.

a) Attach a straight, rigid piece of stainless steel or plastic tubing 3" to 6" long to the flexible tubing exiting from the gradient former.
b) Insert rigid tube until it rests on the bottom.
c) Two bottom clamps should be closed. Using a pipette, fill the mixing chamber with the desired amount of less dense solution for a linearly increasing concentration gradient and pipette the more dense solution into the storage chamber.
d) During filling, the less dense solution enters the tube initially and as the gradient proceeds, the more dense solution raises the less dense solution. The rigid tube remains on the bottom until gradient is complete.
e) When complete, slowly lift the straight tubing out, being careful not to stir the contents. The result is a perfect gradient varying from less dense solution at the top of the tube to the more dense solution at the bottom.
f) Follow steps 8 through 12 (see below).

8) Insert the dual piston assembly into the cylinders using a gentle rocking motion until pistons contact liquid.
9) To dispense the gradient, first close the top piston clamps, then open the two base clamps. Be sure the stir bar is rotating. You are now ready to start your gradient. Start peristaltic pump or depress pistons manually.
10) Before starting a new gradient, empty the slight amount of fluid remaining at the bottom of the gradient former. Tilt the gradient former to position remaining liquid over exit holes.
11) To refill, close the base clamps and open the piston clamps. Remove the piston assembly and refill the chambers with the appropriate solutions. Reinsert the piston assembly and close top clamps.
11) Repeat steps 7 through 12.

2.1 Condensed operating Instructions for the model J17 and J5 Gradient Formers (see sections 2,3 and 4 for more detailed operating instructions and information on the J50 Gradient Former).
Note: Make a practice gradient before attempting final gradient. Refer to Fig. 1.
Note: The leadscrew is provided for manual operation only. To operate with a peristaltic pump, remove the leadscrew. Suction created by the peristaltic pump will draw the pistons downward, keeping fluid levels equal.

USING THE LEADSCREW TO DISPENSE GRADIENT

1) Remove leadscrew assembly from Gradient Former by loosening knurled thumbscrew on piston aluminum brace and by rotating knob counter clockwise. Place leadscrew assembly on bench. Open top clamps, remove dual piston assembly, and place on bench.
2) Close off the two tubing clamps located in the base of Gradient Former. Lubricate cylinders using light film of high vacuum silicone grease (Dow Corning).
3) Place small Teflon-coated stir bar magnet into mixing chamber.
4) Fill the storage chamber with the desired amount of dense solution for a linearly increasing concentration gradient and pipette the less dense solution into the mixing chamber.
5) With base clamps closed and dual piston clamps open, assemble dual piston assembly into cylinders using a gentle rocking motion until piston contacts liquid (or a slight air gap above liquid can be created to push out remaining drops of liquid at end of the fill). Close piston top clamps.
6) Insert leadscrew into Gradient Former until bronze bushing is seated. Secure in place using knurled thumbscrew (do not overtighten, finger tight is adequate).
7) Place Gradient Former on magnetic stirrer and turn on mixer to as high a speed as possible to assure thorough mixing. Place exit tube in position to make gradient.
8) Start gradient by first closing the piston clamps and then opening two base clamps. Rotate the leadscrew clockwise to dispense gradient. A slow, steady rotation of the knob produces best results. You may stop rotating leadscrew at any time, with no harm to the gradient.
9) When gradient is complete, loosen thumbscrew and remove leadscrew assembly completely by rotating counter clockwise. Use leadscrew to dispense gradient only.
10) Close base clamp and open piston clamps. Refill chambers by removing the pistons and filling chambers with appropriate solutions.
11) Repeat Steps 5 through 10.

3.0 THEORY OF EXPONENTIAL GRADIENTS

Concave or convex exponential gradients can be produced using the Jule Gradient Former. For exponential gradient forming, the Gradient Former is arranged as shown in Fig. 3.

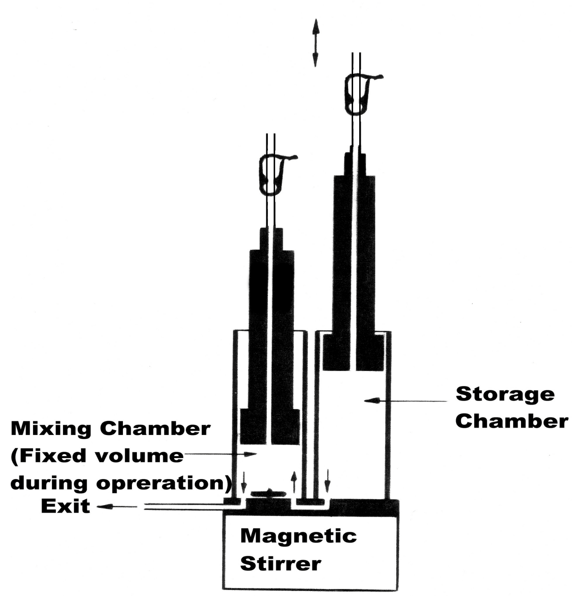

Fig. 3 EXPONENTIAL GRADIENT FORMER

An exponential gradient is produced by withdrawing liquid from the mixing chamber, the volume of which is kept constant. Liquid flows into the mixing chamber from the storage chamber, where the volume is allowed to diminish. If the mixing chamber contains the liquid of lesser concentration, the resulting gradient profile is convex exponential. If the mixing chamber contains the liquid of higher concentration, the resulting gradient profile is concave exponential. The smaller the volume of the mixing chamber, the more convex or concave the gradient. In cases where the gradient volume is large, the storage chamber can be refilled by closing appropriate valves and retracting the piston.

Exponential gradients can be described by the following relationship:

$$Ct = Cr - (Cr - Cm)\, \mathbf{e}^{(-Vt/Vm)}$$

Where:

Ct = Concentration of gradient being delivered at any time "t".
Cr = Starting concentration in storage chamber.
Cm = Starting concentration in mixing chamber.
e = Natural base (i.e., 2.718)
Vt = Volume of gradient withdrawn at time "t".
Vm = Volume of mixing chamber

Fig. 4 shows a few of the numerous concave or convex profiles that can be generated:

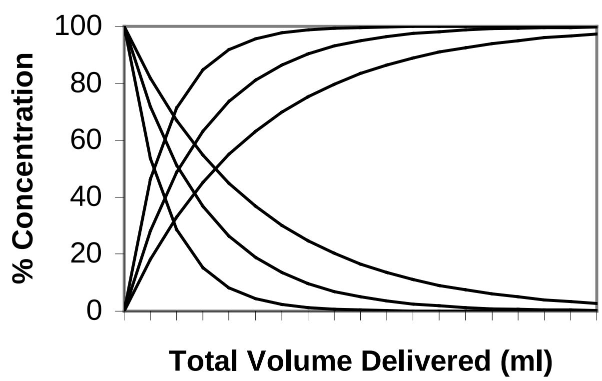

Fig. 4 EXPONENTIAL GRADIENT

Example:

Suppose the mixing chamber contains 10 ml of 0% w/w sucrose (plain water), and the reservoir contains 40% w/w sucrose. This would produce a convex exponential gradient. If one wished to know the concentration of the liquid at the point when 30 ml of gradient has been delivered, it would be as follows, using the equation for exponential gradient:

$$Ct = Cr - (Cr - Cm)\, \mathbf{e}^{(-Vt/Vm)}$$

Where:

$Ct = 40 - (40 - 0)\, \mathbf{e}^{(-30/10)}$
$Ct = 40 - 40\,(2.718)^{-3}$
Ct = 38% w/w sucrose
Or
% gradient concentration = (38/40) x 100 = 95 %

Reference: "Centrifugation in Biology and Medical Science", P. Sheeler.

4.0 ASSEMBLY AND OPERATING PROCEDURE FOR EXPONENTIAL GRADIENTS

Note: For models J17 and J5, the leadscrew assembly is not used to make exponential gradients. Remove leadscrew assembly and follow instructions below.

1) Refer to Fig. 3 to arrange gradient former for exponential gradient forming.
2) Disassemble pistons from brace by loosening set screws in brace near top of piston. Gradient former cylinders are lubricated prior to shipment, but may require lubrication periodically. If cylinders require lubrication coat the inner walls of both cylinders, near the top, with a thin film of high vacuum silicone grease (Dow Corning). Use pistons to spread lubricant over length of cylinder.
3) Close off two downstream clamps located in base of gradient former.
4) Place small Teflon-coated stir magnet into mixing chamber.
5) Using a pipette, fill mixing chamber to desired volume. Use the volume indicator label for reference only. (Refer to Top Filling or Bottom Filling in section 2.0, if necessary.)
6) Open piston clamp to allow air to escape and insert piston into mixing chamber until piston face contacts fluid surface. (To assemble piston, rock it gently to engage O-ring).
7) Close piston clamp.
8) Hold mixing chamber piston in place using a laboratory stand.

   Note: Mixing chamber piston does not move during exponential gradient forming.
9) Fill storage chamber with fluid of higher concentration for a concentration-increasing gradient. No piston is needed for the storage chamber side. If piston is inserted into storage chamber side, leave top clamp open.
10) Place gradient former on a magnetic stirrer and turn on mixer to a high speed to assure thorough mixing.

You are now ready to start an exponential gradient.

11) To start an exponential gradient, open two clamps in base of gradient former (depress storage chamber piston slowly if used). Liquid will exit mixing chamber as an exponential gradient. A very slow flow rate is recommended (2-3 minutes per gradient).

    Note: Once storage chamber is empty, refill by closing the two downstream base clamps and removing the piston to refill the storage chamber. If piston was not used, the storage chamber can be filled any time. The gradient concentration is approximately 80% after two volumes of the mixing chamber has been delivered.
12) If gradient is over and you wish to repeat the gradient, empty fluid in only the mixing chamber by pumping it out. Tilt gradient former to position remaining liquid over exit holes. If desired, rinse by drawing appropriate fluid through exit hole and pumping it out.
13) Refill chambers to desired volumes and repeat exponential gradient.
14) If you wish to restore gradient former to make linear gradients, DO NOT over tighten setscrews, which hold pistons. Just let the setscrews contact the pistons. Test tightness by pulling on pistons.

5.0 Column Chromatography

For controlled low flow rates, attach a pump to the exit port of the gradient former. With piston clamps closed, the piston assembly will be drawn downward during operation, thereby keeping fluid levels equal for perfect linearity. For models J17 and J5, the leadscrew is not used when a pump is attached to the exit port of the gradient former. Always assure fluids are chemically compatible with acrylic.

6.0 Cleaning, Sterilization and Maintenance

Note: Acrylics are unaffected by aqueous solutions of most common laboratory chemicals, by detergents, cleaners, dilute inorganic acids, alkalies and aliphatic hydrocarbons. Plexiglass is not recommended for use with chlorinated or aromatic hydrocarbons. Alcohol’s will cause cylinders, which are a molded-type acrylic, to crack on models J50, J17 and J5.

Note: Sterilize using 3% to 5% sodium hydroxide or sodium hypochlorite (Clorox). Do not autoclave.

Note: When in doubt, consult chemical compatibility chart for acrylic and test using a few drops on outside of cylinder and base. Cylinders on Models J50, J17 and J5 are a molded-type acrylic (extruded). Base and pistons are cast acrylic. Their chemical resistance varies slightly.

1) The Jule Gradient Former is made of sturdy acrylic plastic and may be cleaned by washing with any standard detergent, rinsing thoroughly. Clean the gradient former as soon as possible after using solutions which may gel or polymerize to prevent possible clogging. Care should be taken not to scratch the inside diameter of chambers where pistons are inserted.
2) Use proper size wrench to remove fittings.
3) If removal of piston O-ring for cleaning becomes necessary, use blunt edge, such as spoon handle, to pry O-ring. Grease with a thin coat of silicone-type grease lubricant.
4) Pistons have been machined to fit snugly into cylinders. Use care when cleaning and handling.

7.0 Service (See Warranty)

If the Jule Gradient Former should require service, the following procedure should be used:

1) Call or write to Jule, Inc. to obtain written agreement prior to returning goods.
2) Return defective part(s) to:

Jule, Inc.
185 Research Drive, Unit 6
Milford, CT 06460

Package properly to prevent shipping damage.

3) Include statement of problem or type of service required.
4) Include return address and billing information, if necessary.

8.0 Warranty

The Jule Gradient Former is warranted to be free from defects in materials and workmanship for a period of 90 days from delivery thereof. Jule, inc. will repair or replace and return free of charge any part thereof which is returned to its factory within said period, transportation prepaid by user, and which is found, upon inspection, to have defective in materials or workmanship. This warranty does not include normal wear from use, it does not apply to any product or part thereof which has been altered by anyone other than an employee of Jule, Inc., nor to any product thereof which has been damaged through accident, negligence, failure to follow operating instructions, use beyond the specified capacity of the product, misuse or abuse.

Jule, Inc. reserves the right to change, alter, modify or improve any of its products without any obligation whatever to make corresponding changes to any product previously sold or shipped.

THE FOREGOING OBLIGATIONS ARE IN LIEU OF ALL OTHER OBLIGATIONS AND LIABILITIES INCLUDING NEGLIGENCE AND ALL WARRANTIES, OR MECHANTABILITY OR OTHERWISE, EXPRESSED OR IMPLIED IN FACT OR BY LAW, AND STATE OUR ENTIRE AND EXCLUSIVE LIABILITY AND BUYER'S EXCLUSIVE REMEDY FOR ANY CLAIM OF DAMAGES IN CONNECTION WITH THE SALE OR FURNINSHING OF GOODS OR PARTS, THEIR DESIGN, SUITABILITY FOR USE, INSTALLATION OR OPERATION. JULE, INC WILL IN NO EVENT BE LIABLE FOR ANY SPECIAL OR CONSEQUENTIAL DAMAGES WHATSOEVER, AND THEIR LIABILITY UNDER NO CIRCUMSTANCES WILL EXCEED THE CONTRACT PRICE FOR THE GOODS FOR WHICH LIABILITY IS CLAIMED.

RESISTANCE OF SOME PLASTICS TO CHEMICALS AND STERILIZATION

	Conventional Polyethylene	Linear Polyethylene	Polyallomer	Polypropylene	Polymethyl-pentene	Polytetra-fluoroethylene	Fluoroethylene-Propylene	Chlorotri-fluoroethylene	Polycarbonate	Phenylene Oxide Resin	General Purpose Polystyrene	Styrene-Acrylonitrile	Acrylics	Polyvinyl Chloride	Acetal Resins
Acids, inorganic	E	E	E	E	E	E	E	E	E	E	N	E	E	G	N
Acids, organic	E	E	E	E	E	E	E	E	G	E	G	E	G	G	G
Alcohols	E	E	E	E	E	E	E	E	G	E	G	G	*	G	G
Aldehydes	G	G	G	G	E	E	E	E	F		N	F	F	F	G
Amines	G	G	G	G		E	E	E	N		G	G	G	N	G
Bases, inorganic	E	E	E	E	E	E	E	E	N	E	G	E	E	E	G
Dimethyl sulfoxide (DMSO)	E	E	E	E	E	E	E	E	N		N	N	N	N	
Esters	E	E	E	E	G-N	E	E	E	N		N	N	N	F	G
Ethers	G	G	G	G	N	E	E	E	F		F	N	N	F	E
Foods	E	E	E	E	E	E	E	E	E	E	E	G	G	G	E
Glycols	E	E	E	E	E	E	E	E	G	E	G	G	E	F	E
Hydrocarbons, aliphatic	G	G	G	G	N	E	E	E	F	E	N	F	E	F	G
Hydrocarbons, aromatic	G	G	G	G	N	E	E	E	N	N	N	N	N	N	G
Hydrocarbons, halogenated	G	G	G	G	N	E	E	F	N	N	N	N	N	N	G
Ketones	G	G	G	G	F	E	E	E	N		N	N	N	N	G
Mineral oil	E	E	E	E	E	E	E	E	E	E	G	G	G	E	E
Oils, essential	G	G	G	G	G	E	E	E	G	G	N	F	N	N	
Oils, lubricating	G	E	E	E	E	E	E	E	G	E	G	G	G	E	E
Oils, vegetable	E	E	E	E	E	E	E	E	E	G	G	E	G	E	E
Proteins, unhydrolyzed	E	E	E	E	E	E	E	E	E	E	G	E	G	G	E
Salts	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
Silicones	G	E	E	E	E	E	E	E	E	E	G	G	G	G	
Water	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
Autoclaving	No	Yes[1]		Yes	Yes		Yes		Yes[2]		No	No		No	
Gas	Yes	Yes		Yes	Yes		Yes		Yes		Yes	Yes		Yes	
Dry Heat	No	No		No	No		Yes		No		No	No		No	
Chemical	Yes	Yes		Yes	Yes		Yes		Yes		Yes	Yes		Yes	

Gas: Ethylene oxide Dry heat: 160°C Chemical: Benzalkonium chloride, formalin, ethanol, etc.

E - Excellent. Long exposures (up to one year) at room temperature have no effect. F - Fair. Short exposures at room temperature cause little or no damage under unstressed conditions.
G - Good. Short exposures (less than 24 hours) at room temperature cause no damage. N - Not Recommended. Short exposures may cause permanent damage.
1) Can be autoclaved at 121°C for 20 min. 2) Autoclaving reduces mechanical strength.
Data obtained from the Nalge Company, Rochester, N.Y. and the Modern Plastics Encyclopedia, vol. 49, No. 10A, McGraw-Hill Inc.

*Alcohol will cause cylinders to swell and crack on models J50, J17 and J5.

9.0 Jule Gradient Former Accessories Kit

	Quantity of parts contained in each kit*		
Part Description	**KJ5**	**KJ17**	**KJ50**
Kit price	$ 20	$ 20	$ 20
O-ring seal	2	2	2
Fittings	5	5	5
Tubing (1/16 ID)	6’	6’	6’
Stir Bar	1	1	1
Clamp (Small)	5	5	5

*The contents in each kit may change to accommodate product improvements and design changes.

Gradient Former Manual	$ 6.00
Silicone Grease (5 ounce tube)	$ 20.00

Make checks payable to:

Jule, Inc.
185 Research Drive, Unit 6
Milford, CT 06460
Phone: (800) 648-1772, Fax (203) 878-7448
www.precastgels.com