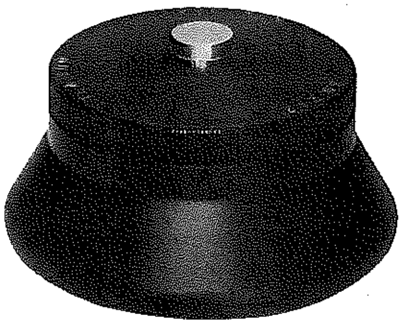
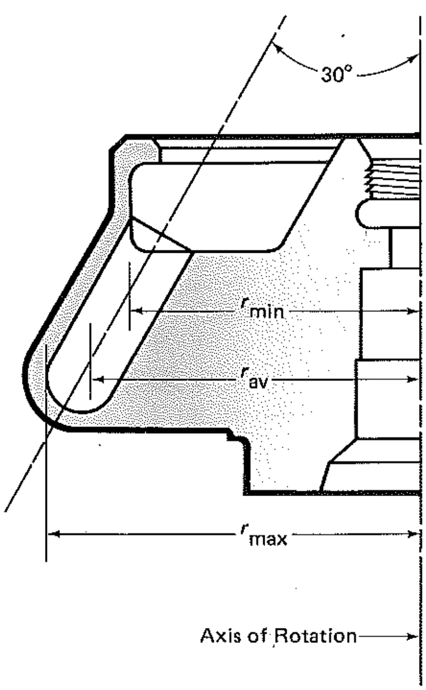
BECKMAN

INSTRUCTIONS FOR USING THE TLA-100 FIXED ANGLE ROTOR

In the Beckman TL-100 Tabletop Ultracentrifuge





U.S. Pat No. 4,372,483
Japanese Pat. No. 1551443
Switzerland Pat. No. 646,881

SPECIFICATIONS

Maximum speed	100 000 rpm
Density rating at full speed	1.7 g/mL
Relative Centrifugal Field* at maximum speed	
At r_{max} (38.9 mm)	436 000 x g
At r_{av} (34.5 mm)	386 000 x g
At r_{min} (30.0 mm)	336 000 x g
k factor at maximum speed	7
Number of tube cavities	20
Nominal tube dimensions	7 x 20 mm
Nominal tube capacity	0.2 mL
Nominal rotor capacity	4 mL
Available tubes	see Table 1
Approximate acceleration time to maximum speed (unloaded rotor)	1 1/2 min
Approximate decleration time from maximum speed (unloaded rotor)	1 min
Weight of fully loaded rotor	0.5 kg (1 lb)
Rotor material	titanium

* Relative Centrifugal Field (RCF) is the ratio of the centrifugal acceleration at a specified radius and speed ($r\omega^2$) to the standard acceleration of gravity (g) according to the following formula:

$$RCF = \frac{r\omega^2}{g}$$

where r is the radius in millimeters, ω is the angular velocity in radians per second (2πRPM/60), and g is the standard acceleration of gravity 9807 mm/s^2). After substitution:

$$RCF = 1.12\, r \left(\frac{RPM}{1000}\right)^2$$

MJM-0589-.2-DT

PUBLISHED BY SPINCO DIVISION OF BECKMAN INSTRUMENTS, INC., PALO ALTO, CALIFORNIA 94304

DESCRIPTION

The TLA-100 fixed angle rotor, rated for 100 000 rpm, is designed to hold up to twenty 7 x 20-mm (5/16 x 3/16-in.) tubes at a 30-degree angle. Used in the TL-100 Tabletop Ultracentrifuge, the rotor generates centrifugal forces for rapid pelleting of small sample volumes, lipoprotein isolation, and isopycnic banding of DNA.

The rotor is made of titanium and is finished with black urethane paint. The lid is made of aluminum and is black-anodized to resist corrosion. The plunger is used to lock the rotor in place on the drive hub before beginning the run. In the lid, two lubricated O-rings made of Buna N maintain atmospheric pressure inside the rotor during centrifugation.

The rotor is specially designed with a fluid-containment annulus, located below the O-ring sealing surface (see Figure 1). The annulus is designed to retain fluid that may escape from leaking or overfilled tubes, thereby preventing the liquid from escaping into the instrument chamber.

For overspeed protection, the TL-100 magnetically identifies rotor speed during the run. This identification system includes a magnetic speed sensor in the rotor chamber of the instrument and magnets on the bottom of the rotor. The overspeed system ensures that the rotor does not exceed its maximum permitted speed. The TLA-100 rotor is warranted for five years (see Warranty).

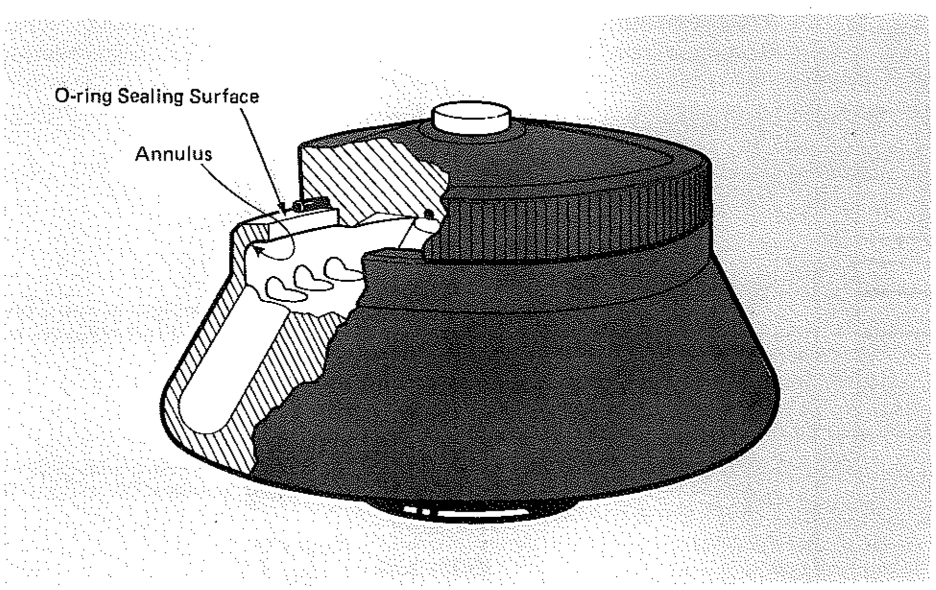


Figure 1. Fluid-Containment Annulus

OPERATION

NOTE: Specific information about the TLA-100 fixed angle rotor is given here. Use the TL-100 instrument instruction manual (TL-IM) in combination with this bulletin for complete ultracentrifuge operation.

TUBES

Available tubes for use in the TLA-100 rotor are listed in Table 1. Be sure to observe the maximum rotor speed limits and use only those tubes listed.

Do not centrifuge any of the tubes below 2°C. Also, do not freeze polyallomer or cellulose propionate tubes before centrifugation, as they may become brittle and crack. Polycarbonate and polyallomer tubes may be centrifuged at temperatures above 25°C, but they should be pretested under anticipated run conditions. Do not run cellulose propionate tubes above 25°C.

The tubes do not need caps. However, be sure to observe the maximum fill volumes listed in Table 1.

Consult publication IN-175 for information on the chemical resistances of rotor and tube materials.

Table 1. Available Tubes (7 x 20 mm; 5/16 x 3/16 in.) for the TLA-100 Fixed Angle Rotor

Description	Part Number (Package of 100)	Maximum Fill Volume (mL)	Maximum Speed (rpm)
thickwall polycarbonate	343775	0.2	100 000
thickwall polyallomer	343621	0.2	80 000
thickwall cellulose propionate	342303	0.2	65 000

ROTOR PREPARATION

Before using the rotor, make sure that the metal threads are lightly lubricated with Spinkote™ lubricant and two O-rings in the lid are lightly coated with silicone vacuum grease. *For runs at other than room temperature, always refrigerate or warm the rotor to the desired temperature beforehand, since titanium is a poor conductor of heat.* If fewer than 20 tubes are being run, they must be arranged symmetrically in the rotor. Opposing tubes must be filled to the same level with liquid of the same density. For convenience, insert the rotor into the portable polypropylene rotor vise. Then place the lid on the rotor and tighten it firmly (clockwise) by hand. No tool is required.

Carefully place the rotor on the drive hub and lock it in place as follows. Gently press the plunger in the rotor lid down until you hear a click. When you remove your finger, the plunger will remain flush with the lid (see Figure 2) if it is properly engaged. If the plunger pops up, repeat the procedure. It is necessary to lock the rotor in place before beginning the run. Consult the TL-100 instrument instruction manual for ultracentrifuge operation.

To release the plunger at the end of the run, press it down into the lid until you hear a click. When you remove your finger, the plunger will pop up to its original position (see Figure 2). Remove the rotor from the instrument and place it in the rotor vise. To remove the rotor lid, unscrew it counterclockwise. Use forceps or a hemostat to remove the tubes.

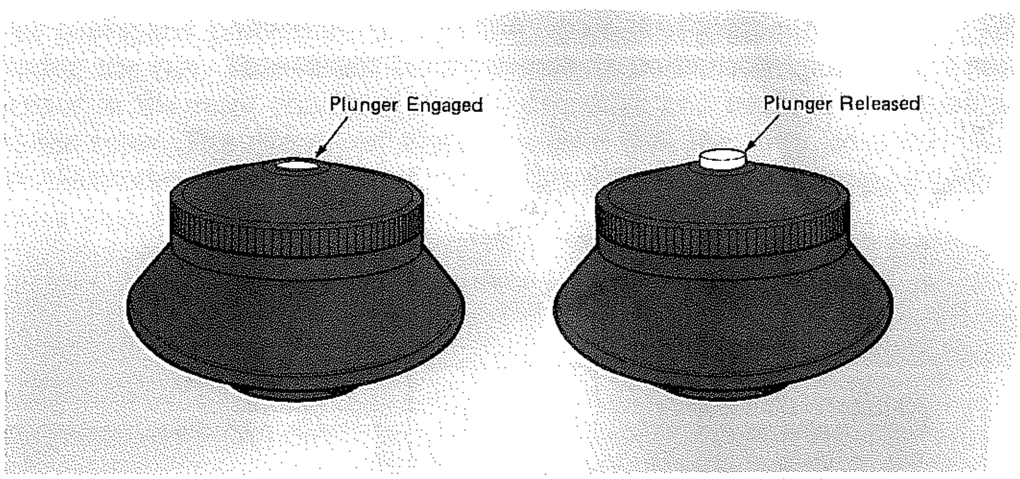


Figure 2. The plunger in locked and released positions in the rotor lid.

RUN TIMES

The *k* factor of the rotor is a measure of the rotor's pelleting efficiency. (Beckman has calculated *k* factors for all of its preparative rotors at maximum rated speed and using full tubes.) The *k* factor is calculated from the formula:

$$k = \frac{\ln (r_{max}/r_{min})}{\omega^2} \times \frac{10^{-10}}{3.6} \quad (1)$$

where ω is the angular velocity of the rotor in radians per second (ω = 0.105 x RPM), r_{max} is the maximum radius, and r_{min} is the minimum radius.

Use the k factor in the following equation to estimate the run time t (in hours) required to pellet particles of known sedimentation coefficient s (in Svedberg units).

$$t = \frac{k}{s} \tag{2}$$

Run times can be estimated for centrifugation at less than maximum speed by adjusting the k factor as follows:

$$k_{adj} = k \left(\frac{\text{rated speed of rotor}}{\text{run speed}} \right)^2 \tag{3}$$

Run times can also be estimated from data established in prior experiments if the k factor of the previous rotor is known. For any two rotors a and b:

$$\frac{t_a}{t_b} = \frac{k_a}{k_b} \tag{4}$$

where the k factors have been adjusted for the actual run speed used.

RUN SPEEDS

The centrifugal force at a given radius in a rotor is a function of run speed. Comparisons of forces between different rotors are made by comparing the rotors' relative centrifugal fields (RCF). When rotational speed is selected so that identical samples are subjected to the same RCF in two different rotors, one may then describe the samples as being subjected to the same force (see Table 2). Rotational speeds may not be

Table 2. Relative Centrifugal Fields. Entries in this table are calculated from the formula RCF = 1.12 r (RPM/1000)² and then rounded to three significant digits.

Rotor Speed (rpm)	Relative Centrifugal Field (x g)			k Factor*
	At r_{max} (38.9 mm)	At r_{av} (34.5 mm)	At r_{min} (30.0 mm)	
100 000	436 000	386 000	336 000	7
95 000	393 000	349 000	303 000	7
90 000	353 000	313 000	272 000	8
85 000	315 000	279 000	243 000	9
80 000	279 000	247 000	215 000	10
75 000	245 000	217 000	189 000	12
70 000	213 000	189 000	165 000	13
65 000	184 000	163 000	142 000	16
60 000	157 000	139 000	121 000	18
55 000	132 000	117 000	102 000	22
50 000	109 000	96 600	84 000	26

* Calculated for all Beckman preparative rotors as a measure of the rotor's relative pelleting efficiency in water at 20°C.

selected in excess of the limits provided in Table 1. These speeds may also need to be reduced when high-density solutions are centrifuged or when salts that may precipitate (e.g., CsCl) are run.

For solution densities greater than 1.7 g/mL in this rotor, use equation (5) to calculate maximum allowable rotor speed.

$$\text{RPM} = (100\ 000\ \text{rpm})\sqrt{\frac{1.7\ \text{g/mL}}{\text{density of tube contents}}} \quad (5)$$

When CsCl or other self-forming-gradient salt is centrifuged, this equation will not usually guard against the precipitation of salt crystals. Figures 3 and 4, together with the description and samples below, show how to reduce run speeds when using CsCl gradients.

> **NOTE**: The curves in Figures 3 and 4 are for solutions of CsCl only. If other salts are present in significant concentrations, the overall CsCl concentration of salts must be reduced. This prevents precipitation of salts that concentrate at the tube bottom.

Solid CsCl has a density of 4 g/mL, and if precipitated during centrifugation may cause rotor failure. Precipitation will also alter density distribution, and therefore sample separation. In general, lower speeds provide better resolution, but longer run times will be required to achieve particle equilibrium. Curves are provided up to the maximum rated speed of the rotor; however, *tubes must never be centrifuged faster than the limits given in Table 1.* Speed and CsCl density combinations in Figure 3 that intersect on or below the curves ensure that CsCl will not precipitate during centrifugation. Curves are provided at two temperatures: 20°C (the black curves) and 4°C (the gray curves).

SELECTING CsCl GRADIENTS

Rotor speed determines the slope of a CsCl gradient (Figure 4), and must be limited so that CsCl precipitation is avoided (see RUN SPEEDS, above). The reference curves in Figure 4 show gradient distributions at equilibrium. Each curve in Figure 4 is within the density limits allowed for the TLA-100 rotor; each curve was generated for a single run speed using the maximum allowable homogeneous CsCl densities (one for each fill level) that avoid precipitation at that speed.[1] Figure 4 can also be used to approximate the banding positions of sample particles.

[1] The gradients in Figure 4 can be generated from discontinuous gradients or from homogeneous solutions. But the total concentration of CsCl in solution must be equivalent to a homogeneous solution corresponding to the curves in Figure 3.

ADJUSTING FILL VOLUMES

Several fill volumes are possible in a tube. If a tube is only partially filled with gradient solution, however, mineral oil (or some other low-density, immiscible liquid) must be floated on the tube contents to fill the tube to its maximum volume. Note that at a given speed, as the fill level is decreased, the homogeneous CsCl concentration required to generate a given density distribution increase.

For example, a 1/4-filled tube of 1.81 g/mL homogeneous CsCl solution at 20°C may be centrifuged at 90 000 rpm (see Figure 3). The segment of the 90 000 curve (Figure 4) from the 1/4-filled line to 1.86 g/mL at the tube bottom represents this gradient. The same solution in a 1/2-filled tube (Figure 3) may be centrifuged no faster than 74 000 rpm. Interpolate in Figure 4 between the 70 000 rpm and 80 000 rpm curves and draw the new 74 000 rpm gradient curve to the 1/2-filled level. The same solution in a 3/4-filled tube may be centrifuged at 66 000 rpm; Figure 4 presents the gradient profile (use the 3/4-filled segment only). A tube *full* of the 1.81 g/mL CsCl solution may be centrifuged no faster than 60 000 rpm.

TYPICAL EXAMPLES FOR DETERMINING CsCl RUN PARAMETERS

Example A: Knowing homogeneous CsCl solution density (e.g., 1.70 g/mL) and approximate particle densities (e.g., 1.70 and 1.65 g/mL), where will particles band?

1. In Figure 3, find the curve that corresponds to the desired run temperature (e.g., 20°C) and tube fill level (e.g., full). The rotor speed (from the abscissa) corresponding to the point where this curve and the homogeneous CsCl density intersect is the maximum allowable run speed (e.g., 100 000 rpm).
2. In Figure 4, sketch in a horizontal line corresponding to each particle density.
3. Mark the point in the figure where each particle density intersects the curve corresponding to the selected run speed (100 000 rpm) and temperature.
4. Particles will band at these locations along the tube length.

Thus, according to Figure 4, the particles will band about one-half to three-quarters of the way from the tube bottom (33 and 31.5 mm from the axis of rotation, respectively). Thus, in the rotor (during centrifugation) they will be about 1.5 mm apart. Since the volume between the bands remains constant, when the tube is held upright the bands will reorient to about 3 mm apart.

Note that since both particle classes band in the lower half of the tube contents, the samples might be run in 3/4-filled tubes.

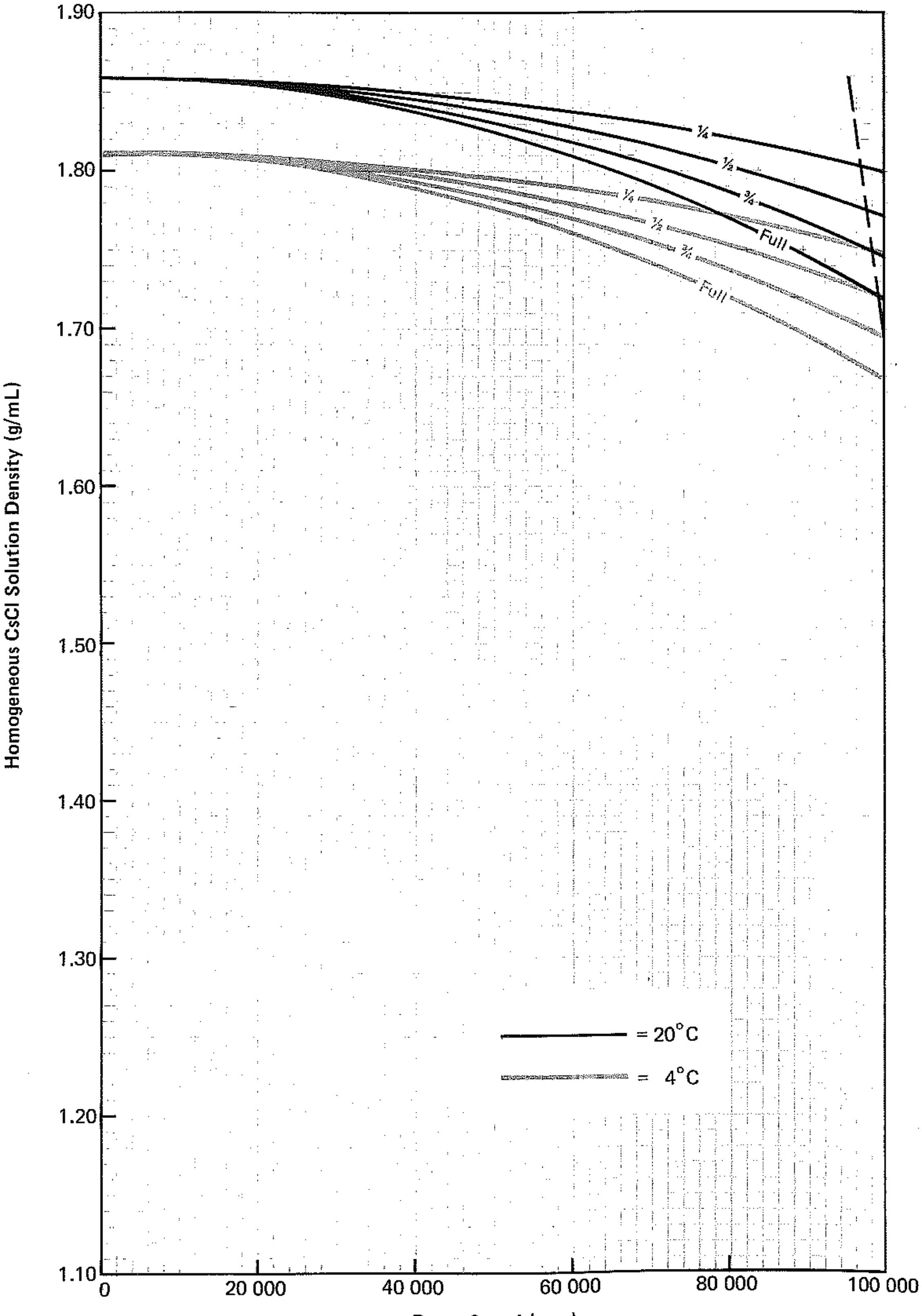
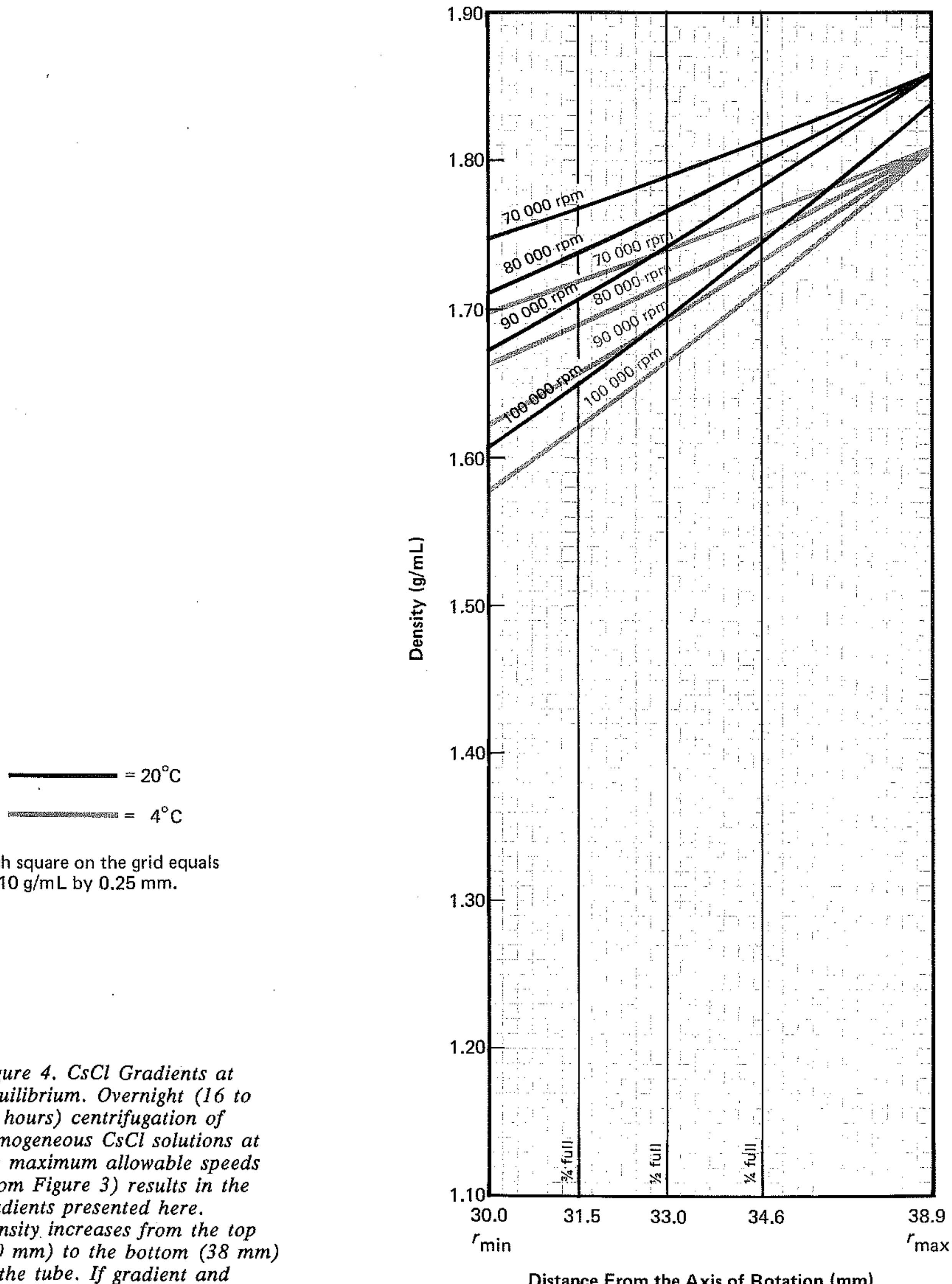


Figure 3. Precipitation Curves. Using combinations of rotor speeds and homogeneous CsCl solution densities that intersect on or below these curves ensures that CsCl will not precipitate during centrifugation. If gradient and sample solutions do not fill the tube to its maximum fill volume, add mineral oil to fill.

The dashed line is a representation of equation (5), and is shown here to illustrate the inability of that equation to guard against CsCl precipitation.



Each square on the grid equals 0.010 g/mL by 0.25 mm.

Figure 4. CsCl Gradients at Equilibrium. Overnight (16 to 18 hours) centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 3) results in the gradients presented here. Density increases from the top (30 mm) to the bottom (38 mm) of the tube. If gradient and sample solutions do not fill the tube to its maximum fill volume, add mineral oil to fill.

Example B: Knowing particle densities (e.g., 1.720 and 1.750 g/mL), how do you get the best separation?

1. In Figure 4, sketch in a horizontal line corresponding to each particle density.

2. Select the curve at the desired temperature (e.g., 20°C) and tube volume (e.g., full) that gives the best particle separation.

3. Note the run speed indicated along the selected curve (e.g., 90 000 rpm).

4. From Figure 3 select the maximum[2] homogeneous CsCl density (e.g., 1.74 g/mL) that corresponds to the temperature, run speed, and fill level established above. These parameters will provide the particle banding pattern selected in Figure 4.

In this example, particles of densities 1.720 and 1.750 g/mL will, in Figure 4, separate better along the 90 000 rpm curve than along the 100 000 rpm curves. At 90 000 rpm, bands will form at about 32 mm and 33 mm from the axis of rotation (about one-half of the way from the tube bottom). This 1-mm separation in the rotor (during centrifugation) will reorient to about a 2.0-mm separation when the tube is removed and is held upright. If you want to run a 3/4-filled tube generating that portion of the gradient represented by the curves in Figure 4, between 31.5 mm and r_{max} , refer to the 3/4-full curves in Figure 3. For the example mentioned above, with 90 000 rpm as the run speed, use 1.765 g/mL as your starting density.

SAMPLE RECOVERY SYSTEM

The Beckman CentriTube Slicer is designed to recover sample fractions from small plastic tubes. Contact your Beckman Representative for detailed information on the Slicer.

MAINTENANCE

Store the rotor in a dry environment (not in the instrument) with the lid removed. Store the lid upside down to protect the plunger fingers from getting damaged. If the plunger gets damaged, contact your Beckman Field Service Representative regarding its repair or replacement.

Routinely inspect the O-rings in the rotor lid. Replace them about twice a year or whenever they are worn or damaged. (Do not use sharp tools on

[2] To increase band separation, run at lower speeds using the maximum allowable CsCl concentration for that speed. Running at a lower speed, however, may increase run time.

the rotor.) Apply silicone vacuum grease to the O-rings before each run. Keep the threads of the rotor assembly well lubricated with Spinkote lubricant. Refer to publication IN-175 for the chemical resistances of rotor and tube materials. Your Beckman Representative provides contact with the Field Rotor Inspection Program and the rotor repair center.

RETURNING A ROTOR

Before returning a rotor or accessory for any reason, prior permission (a Returned Goods Authorization form) must be obtained from Beckman Instruments. This RGA form may be obtained from your local Sales Representative. It should contain the following information:

- serial number,
- a history of use (approximate frequency of use),
- the reason for the return,
- the original purchase order number, billing number, and shipping number, if possible,
- the name and phone number of the person to be notified upon receipt of the rotor or accessory at the factory, and
- the name and phone number of the person to be notified about repair costs, etc.

To protect our personnel, it is the customer's responsibility to ensure that the parts are free from pathogens and/or radioactivity. Sterilization and decontamination must be done before returning the parts. Smaller items (such as tubes, bottles, etc.) should be enclosed in a sealed plastic bag.

All parts must be accompanied by a note, plainly visible on the outside of the box or bag, stating that they are safe to handle and that they are not contaminated with pathogens or radioactivity. Failure to attach this notification will result in return or disposal of the items without review of the reported problem.

Use the address label printed on the RGA form when mailing the rotor and/or accessories to:

Spinco Division
Beckman Instruments, Inc.
1050 Page Mill Road
Palo Alto, CA 94304

Attention: Returned Goods

SUPPLY LIST

TLA-100 fixed angle rotor	343840
Tubes	see Table 1
Beckman Centrifuge Slicer Kit	350745
7-mm tube rack	348302
Lid assembly	343845
O-ring (outer, rotor lid)	824953
O-ring (inner, rotor lid)	824412
Rotor vise assembly	346133
Forceps	878446
Spinkote lubricant	306812
Silicone vacuum grease	335148
Rotor cleaning brush	347404
Beckman Solution 555	339555