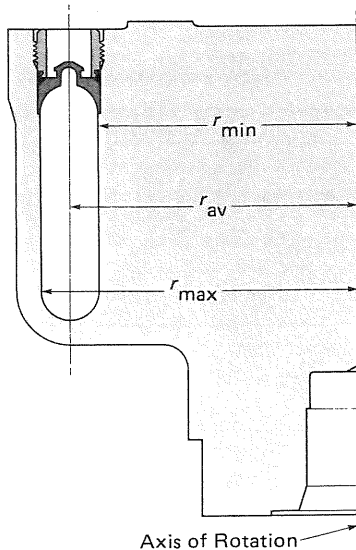
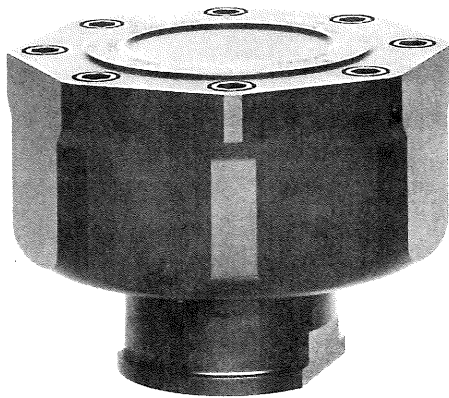


serial # 988

INSTRUCTIONS FOR USING THE VTi 80 VERTICAL TUBE ROTOR

In Beckman Class H and R Preparative Ultracentrifuges



U.S. Pat. Nos. 4,102,490; 4,290,550; 4,235,367
Japanese Pat. Nos. 1,469,154; 1,457,500

SPECIFICATIONS

Maximum speed	80 000 rpm
Density rating at full speed	1.7 g/mL
Relative Centrifugal Field* at maximum speed	
At r_{max} (71.1 mm) 71.1	509 644 x g
At r_{av} (64.5 mm)	462 336 x g
At r_{min} (57.9 mm)	415 027 x g
k factor at maximum speed	8
Number of tube cavities	8
Available tubes	see Table 1
Nominal dimensions of largest tubes	1/2 x 2 in. (13 x 51 mm)
Nominal tube capacity	5.1 mL
Nominal rotor capacity	40.8 mL
Approximate acceleration time to maximum speed (rotor fully loaded, in the L8-80M ultracentrifuge)	6.5 min
Approximate deceleration time from maximum speed (rotor fully loaded, in the L8-80M ultracentrifuge, brake on)	3.5 min
Weight of fully loaded rotor	6 kg (13 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 ($2\pi\text{RPM}/60$), and g is the standard acceleration of gravity (9807 mm/s^2). After substitution:

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

DESCRIPTION

The VTi 80 vertical tube rotor, rated for 80 000 rpm, is designed to centrifuge up to eight ½-in. diameter Quick-Seal[®] tubes in an upright position. Used in class H and R preparative ultracentrifuges, the rotor develops centrifugal forces that can efficiently band DNA or proteins in small volumes. Up to 40.8 mL of gradient and sample can be centrifuged per run.

The rotor is made of titanium and is finished with black urethane paint. A plug, tube spacer, and, if necessary, a tube adapter, hold each tube in the rotor (Figure 1); a Hytrel¹ gasket forms a closure around each plug. Plugs and spacers are black-anodized aluminum; adapters are Noryl plastic. Because of the weight of the rotor, there are no drive pins in the rotor drive hole.

A photoelectric detector in the ultracentrifuge monitors the overspeed disk on the bottom of the rotor (see the Supply List) and shuts down the run if speeds exceeding 80 000 rpm are registered. The VTi 80 rotor is warranted for 5000 runs, 10 000 hours of centrifugation, or 5 years, whichever occurs first (see the Warranty).

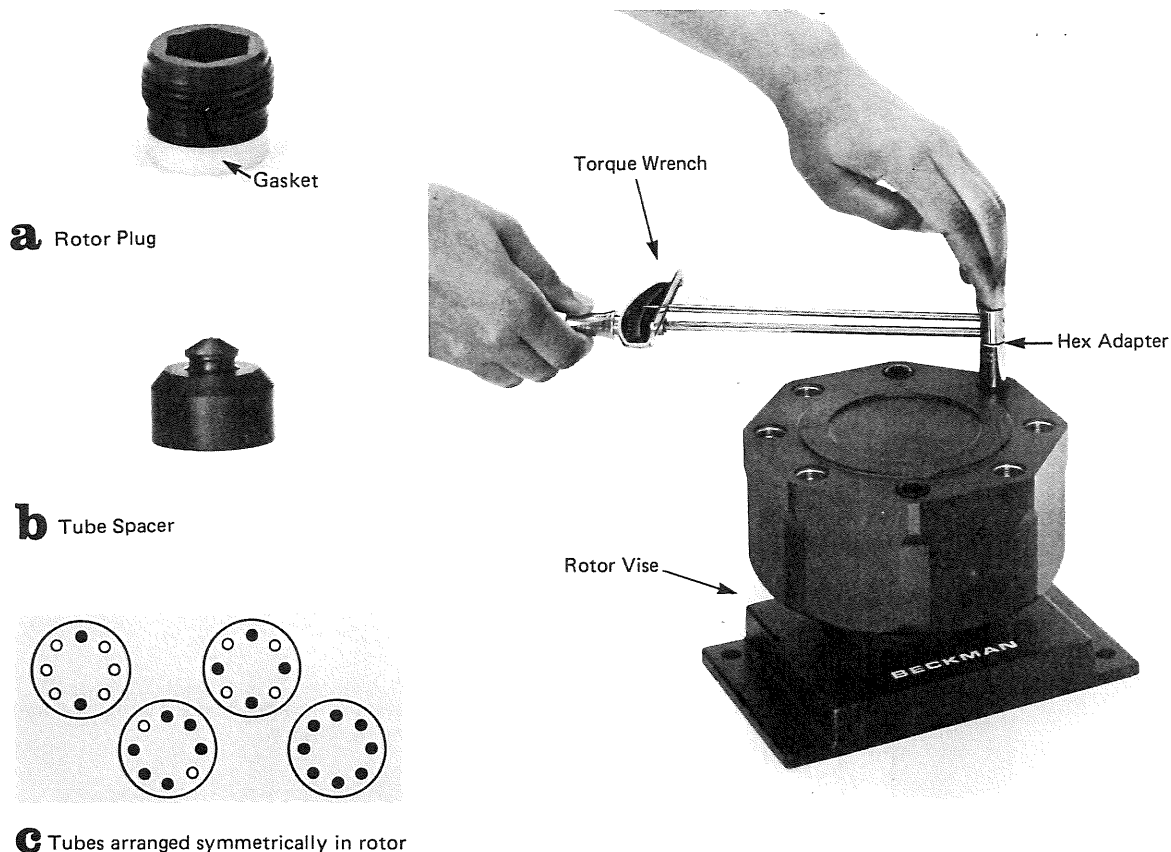


Figure 1. The VTi 80 Rotor. Required parts for use of the rotor are shown, as well as acceptable loading patterns. The tube sealing kit (see SUPPLY LIST) is also required.

¹ A registered trademark of E.I. du Pont de Nemours & Company.

OPERATION

NOTE: Specific information about the VTi 80 rotor is given here. Information common to this and other rotors is contained in the Rotors and Tubes Manual, LR-1M, which should be used together with this bulletin for complete rotor and accessory operation.

The VTi 80 rotor may only be operated with ½-in. diameter Quick-Seal tubes (see Table 1). Polyallomer tubes have been tested for use at temperatures between 2 and 25°C. Do not freeze polyallomer tubes before centrifugation, as they may become brittle and crack. Ultra-Clear™ tubes have been tested for use at temperatures between 4 and 20°C. For use at other temperatures, pretest both kinds of tubes under anticipated run conditions. Ultra-Clear tubes should not be autoclaved or used with solutions of pH greater than 8. Consult the Rotors and Tubes Manual or publication IN-163 or IN-181 for detailed information on the use and care of the Quick-Seal tubes.

Table 1. Available Quick-Seal Tubes for the VTi 80 Rotor

Dimensions (in./mm)	Volume (mL)	Material	Part Number	Spacer	Adapter	Rack †
½ x 2 in. 13 x 51 mm	5.1	polyallomer	342412	342883	---	342423
½ x 2 in. 13 x 51 mm	5.1	Ultra-Clear	344075	342883	---	342423
½ x 1 in. 13 x 25.5 mm	2	polyallomer	345829	342883	345827*	342488

*The adapter is made of Noryl, a registered trademark of General Electric.

†The small 1-in. tube must be sealed, using the 5/8 x 3-in. tube rack (342488) in which the rack adapter, 345832, is used if the tube-sealer rack is used. If using the Tube Topper, use the tube rack 348122 (24 places), which is designed for any ½-in. tube.

ROTOR PREPARATION AND USE

NOTE: Do not run an empty rotor. Place filled tubes in at least two opposing cavities.

Before using the rotor, make certain the overspeed disk is properly attached to the bottom of the rotor. If it is missing, replace it according to the instructions in the Rotors and Tubes Manual. Be sure that the threads in the rotor cavities are well lubricated with Spinkote™ lubricant. For runs at other than room temperature, always refrigerate or warm the rotor beforehand, since titanium is a poor conductor of heat.

Load the filled and sealed tubes symmetrically into the rotor. Tubes placed opposite each other in the rotor should be filled with liquid of the same density.

Set the rotor into the vise, which should be bolted or clamped to a rigid surface.

In a vertical tube rotor, it is important that each cavity being used is completely filled.

Therefore, load cavities exactly as shown in Figure 2. Inspect the plugs and gaskets for damage—the high forces generated in this rotor can cause damaged components to fail. Lubricate the plug threads with Spinkote; plug gaskets do not require lubrication. Then insert a plug (gasket-end down) over each spacer and screw it in—do not insert plugs into empty cavities. Leave unused cavities empty. *Tighten each plug to 120 inch-*

pounds (13.6 N·m) as shown in Figure 1, using the torque wrench and hex adapter. Be careful not to overtighten the plugs. The rotor is now ready to be placed into an instrument for centrifugation. Consult the appropriate instrument instruction manual for ultracentrifuge operation.

Vertical banding of sample and gradient occurs during centrifugation. With deceleration, tube contents reorient back to a horizontal position. For gradient stability when *performed gradients* are used, acceleration of the centrifuge drive should be slowed by doing one of the following:

- in the L8M series of ultracentrifuges, select a slow ACCEL profile.
- in the L8 series of ultracentrifuges, select SLOW ACCEL.
- In the L5 and L5B series of ultracentrifuges without Slow Acceleration Accessories (SAA), set the acceleration dial on "1" (or SLOW) until the tachometer reads 2000 rpm. Then turn the dial to "10" (or FAST). For the stability of shallow performed sucrose gradients of less than 5 to 20% (wt/wt), use of the SAA is recommended.

- in the L5 and L5B series of ultracentrifuges with SAA, use the SAA.

For the stability of gradients during deceleration, do one of the following:

- in the L8M series of ultracentrifuges, select a slow DECEL profile.
- in the L8 series of ultracentrifuges, select SLOW ACCEL (brake will be released at 750 rpm).
- in the L7 ultracentrifuges, set the brake switch at the 800 rpm position.
- in the L5B series of ultracentrifuges, set the BRAKE switch on SLOW or use the SAA (brake will be released between 1500 and 1000 rpm).
- in the L5 series of ultracentrifuges the brake may be turned off manually just as the rotor decelerates past 2000 rpm. Leaving the brake off from the beginning of the run is not recommended as deceleration will take a long time.

After a run, remove the plugs with the torque wrench. If plastic adapters were used, remove them with the 338765 tool. Remove the spacers and tubes with a hemostat or tube removal tool part number 342419.

CAUTION

If disassembly reveals evidence of leakage, and pathogenic or radioactive materials are involved, the operator should assume that some fluid escaped the rotor. Appropriate decontamination procedures should be applied to the centrifuge and accessories.

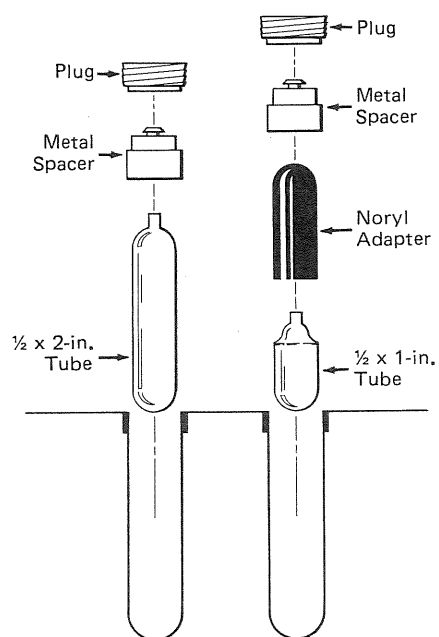


Figure 2. Loading the Rotor with the Correct Combination of Adapter and/or Spacer

RUN TIMES

Sedimentation velocity (rate zonal) run time t can be estimated from data established in prior rate zonal experiments if the k factor of the previous rotor is known.² For any two rotors³ a and b:

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

where k_a and k_b have been adjusted: $k_{\text{actual}} = k_{\text{rated}} \left(\frac{\text{rated speed}}{\text{actual speed}} \right)^2$. (1)

Equilibrium sedimentation (isopycnic gradient) run times should *not* be calculated using k factors. CsCl gradients, for example, generally require overnight centrifugation.

RUN SPEED

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 adjusted so that identical samples are subjected to the same RCF in two different rotors, one may then describe the samples as having been subjected to the same force. The RCF at a number of rotor speeds is provided in Table 2.

Table 3. Relative Centrifugal Fields. Entries in this table are calculated from the formula $RCF = 1.12 r (RPM/1000)^2$.

Rotor Speed (rpm)	Relative Centrifugal Field (x g)			k Factor*
	At r_{max} (71.1 mm)	At r_{av} (64.5 mm)	At r_{min} (57.9 mm)	
80 000	509 644	462 336	415 027	8
75 000	447 930	406 350	364 770	9
70 000	390 196	353 976	317 755	11
65 000	336 445	305 214	273 982	12
60 000	286 675	260 064	233 452	14
55 000	240 886	218 526	196 165	17
50 000	199 080	180 600	162 120	21
45 000	161 254	146 286	131 317	26
40 000	127 411	115 584	103 756	33

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

If solutions more dense than 1.7 g/mL are centrifuged in this rotor, the maximum allowable run speed must be reduced according to the following equation:

$$\text{reduced maximum speed} = (80\,000 \text{ rpm}) \sqrt{\frac{1.7 \text{ g/mL}}{\text{density of tube contents}}} \quad (2)$$

Further speed limits must be imposed when CsCl or other self-forming gradient salts are centrifuged, as equation (2) does not predict concentration limits that are required to avoid

² Clearing factor k is calculated for all Beckman preparative rotors as a measure of the rotor's relative pelleting efficiency in water at 20°C.

³ The method is most accurate when comparing two like rotors, i.e., two vertical tube rotors or two swinging bucket rotors.

precipitation of salt crystals. Precipitation during centrifugation would alter the density distribution of CsCl and this would change sample separation. Figure 3, together with the description and examples below, shows how to reduce run speeds when using CsCl gradients.

SELECTING CsCl GRADIENTS

CAUTION

Although this rotor is rated for densities of 1.7 g/mL, a 1.7 g/mL CsCl solution may not be centrifuged at 80 000 rpm in it because CsCl will precipitate. See Figure 3a for allowable CsCl densities at various speeds.

Rotor speed is used to control the slope of CsCl density-equilibrium gradients (Figure 3). Speed must be limited, however, to avoid salt precipitation (see **RUN SPEED**, above). Figure 3a gives the CsCl concentration-limiting curves for full tubes.

NOTE: The curves in Figure 3 are for solutions of CsCl salt only. If other salts are present in significant concentrations, the overall CsCl concentration or the rotor speed must be reduced. This prevents precipitation of salts that concentrate along the side of the tube.

Figure 3b gives the equilibrium gradients that result from centrifugation using the maximum densities allowed by Figure 3a at several run speeds. Note that at the lower speeds, longer run times will be required to achieve particle equilibrium. Figure 3c gives the gradients that result from centrifugation using lower-than-maximum-allowable CsCl concentrations. These reduced-density curves can be used to make particles band more towards the middle of a tube, where volume between bands will be the greatest.

The gradients in Figures 3b and 3c can be generated from step or linear gradients, or from homogeneous solutions. But the total concentration of CsCl in solution must be equivalent to the homogeneous concentrations specified.

TYPICAL EXAMPLES FOR DETERMINING CsCl RUN PARAMETERS

Example A: Knowing homogeneous CsCl solution density (e.g., 1.65 g/mL) and approximate particle densities (e.g., 1.7 and 1.65 g/mL), where will particles band?

1. At 20°C, according to Figure 3a, a solution of 1.65 g/mL should be centrifuged no faster than 75 000 rpm.
2. In Figure 3b, sketch in a horizontal line corresponding to each particle density.
3. Mark the point in Figure 3b where each particle density intersects the curve corresponding to the run speed (75 000 rpm) and temperature (20°C).
4. Particles will band at these marked points across the tube diameter (lower axis of Figure 3b) at equilibrium during centrifugation. After centrifugation the bands will reorient (top axis).

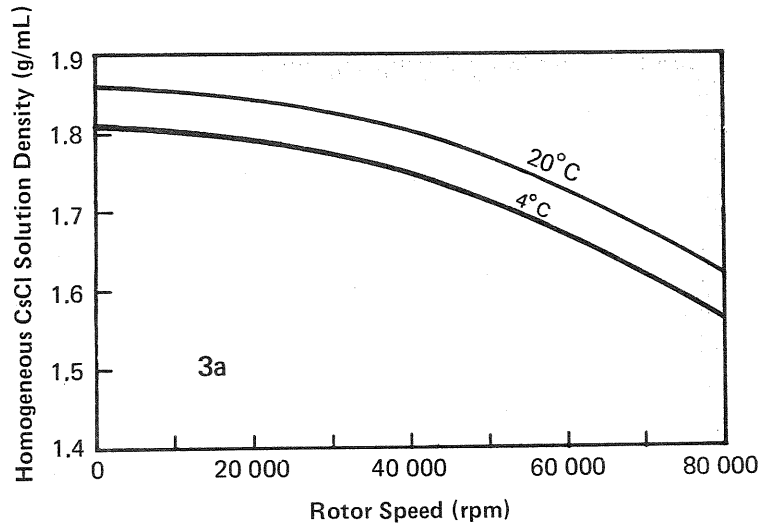
If the desired gradient curve is not presented in Figure 3b, interpolate between the nearest curves and draw it in. Thus, for this example, a 75 000 rpm curve should be drawn in between the 70 000 and 80 000 rpm curves. Particles will band along this curve about one half and one third of the way from the right edge of the figure. Using the horizontal axis, it can be estimated that these particles will be about 1½ mm apart at equilibrium during centrifugation. They will be separated by ¾ mL (top axis).

Figure 3. CsCl Precipitation and Equilibrium Curves

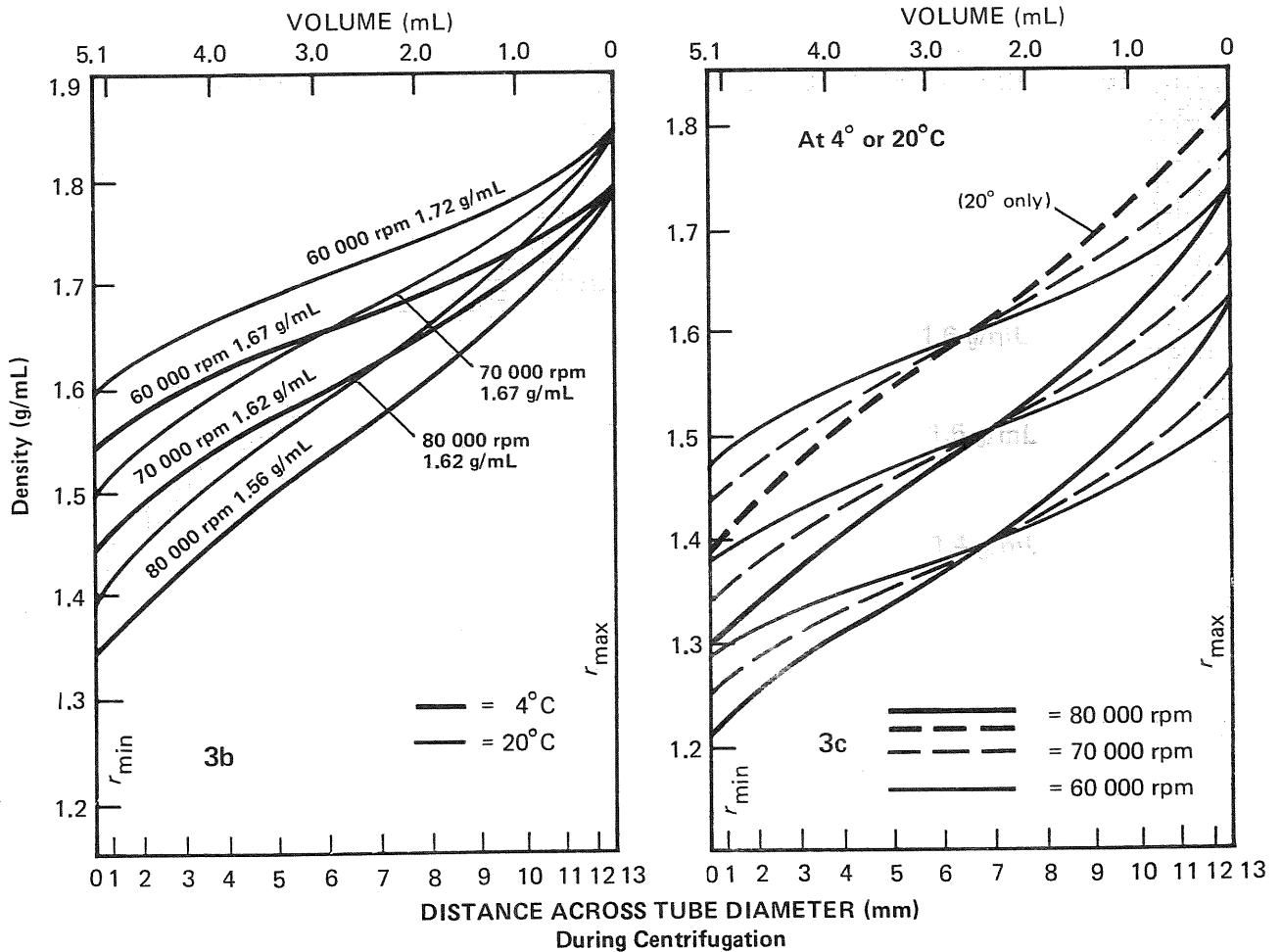
a. *Precipitation Curves.* Using combinations of rotor speeds and homogeneous CsCl solution densities that intersect on or below these curves ensures that CsCl will not precipitate during centrifugation.

b. *Equilibrium Gradient Curves.* Centrifugation of homogeneous CsCl solutions at the maximum allowable speeds (from Figure 3a) results in the gradients presented here. Black curves are for 20°C. Gray curves are for 4°C. The homogeneous CsCl solution density used to generate each curve is printed along the curve.

c. *Equilibrium Gradients for Lower Densities.* Centrifugation of less-than-maximum-allowable-density solutions may sometimes give better particle separations. Densities used to generate curves are printed along the curves. Note that at 4°C, only two curves (60 000 rpm and 70 000 rpm) are shown for 1.6 g/mL because CsCl would precipitate at 80 000 rpm.



AT EQUILIBRIUM
During or After Centrifugation



Example B: Knowing particle densities (e.g., 1.600 and 1.610 g/mL), how do you get the best separation? Assume 20°C operation.

1. In Figure 3b or 3c sketch in a horizontal line corresponding to each particle density.
2. Select the curve that gives the best particle separation at the desired temperature. Particles will band at points across the tube diameter where the sketched lines intersect this curve (lower axis) at equilibrium during centrifugation. After centrifugation the bands will reorient (top axis).
3. Note the run speed and homogeneous CsCl concentration for the selected curve. (The CsCl concentration required is printed on or along each curve.)

In this case (20°C), the 1.6 g/mL, 60 000 rpm curves in Figure 3c give the best separation. The 60 000 rpm curves under maximum density conditions in Figure 3b are above the density range that would give good separation, and at the higher speeds in Figure 3b, the steeper gradients give less distance between particle bands.

In Figure 3c the 1.6 g/mL, 60 000 rpm curves intersect the particle densities in such a way that particles band about ½ mm apart at equilibrium during centrifugation (the lower axis). They are separated by ¼ mL (upper axis).

MAINTENANCE

- Routinely inspect the overspeed disk on the bottom of the rotor. If it is damaged, replace it (see the Rotors and Tubes Manual).
- Occasionally lubricate the metal threads in the rotor cavities with a thin coat of Spinkote lubricant. Failure to keep these threads lubricated can result in seized or galled threads.
- The rotor plug gasket requires no maintenance, but should be replaced if damaged. In general, sharp tools or brushes that may scratch the rotor surface should not be used. However, to replace the gasket a razor blade must be used to cut the gasket from the plug. *Do this carefully so that the plug is not damaged.* The new gasket snaps onto the grooved end of the plug.

Store the rotor in a dry environment (not in the instrument). Refer to the Rotors and Tubes Manual or publication IN-175 for the chemical resistances of rotor or tube materials. Your Beckman Representative provides contact with the Field Rotor Inspection Program and the rotor repair center.

CLEANING

Wash the rotor occasionally to prevent buildup of residues on the rotor. If salts or other corrosive materials have been run, or if spillage has occurred, wash the rotor and rotor components immediately. Do not allow corrosive solutions to dry on the rotor or in plug threads. The Rotor Cleaning Kit (see **SUPPLY LIST**) contains two brushes that will not scratch and two quarts of mild, Solution 555™ detergent for use with rotors, tubes, and accessories. Solution 555 should be diluted 5 or 10 to 1 with water. Rinse the cleaned rotor with distilled water and air-dry upside down.

If the rotor or tubes are contaminated with radioactive solutions, appropriate decontamination procedures should be followed. Check the chemical resistances list in the Rotors and Tubes Manual to be sure the decontamination method will not damage any part of the rotor.

STERILIZATION

All rotor components may be sterilized by autoclaving at 121°C for one hour. To autoclave, remove the plugs from the rotor and place the rotor, spacers, and plugs in the autoclave with the rotor upside down. Rotor components may be disinfected with 70% ethanol.⁴

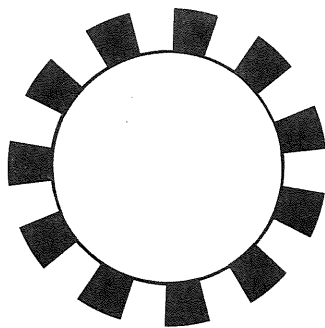
SUPPLY LIST

Replacement Rotor Supplies

VTi 80 rotor	342885
Quick-Seal tubes, spacers, adapters, and racks	see Table 1
Rotor plug	342881
Plug gasket	342882
Torque wrench.	858121
Hex adapter	342887
Plastic adapter removal tool.	338765
Overspeed disk (80 000 rpm)	341965
Rotor vise assembly	342705
Tube removal tool	342419

Other

Sample application block	342694
Ultra-Clear tube sealing agent	345395
Spinkote lubricant	306812
Rotor cleaning kit	339558
Beckman Solution 555.	339555
Tube sealing kit (with 60 Hz, 120 V sealer)	342429
Tube sealing kit (with 50 Hz, 220 V sealer)	342424
Quick-Seal Tube Topper (60 Hz)	348137
Quick-Seal Tube Topper (50 Hz)	349647
Beckman Fraction Recovery System	343890



*Overspeed Disk for the
VTi 80 Rotor (22 sectors)*

⁴ Flammability hazard. Do not use in or near operating ultracentrifuges.