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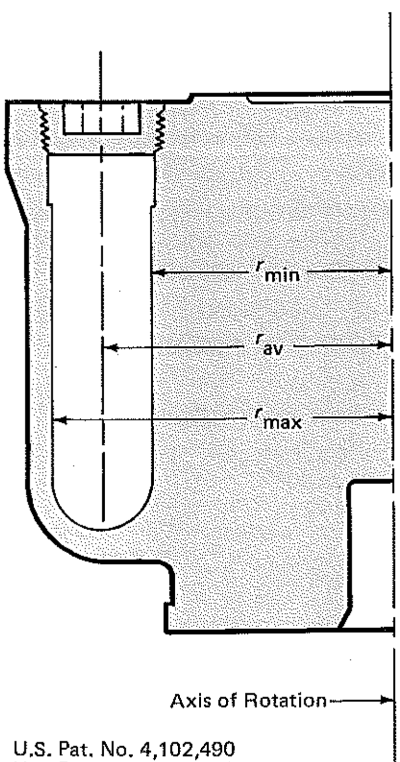
BECKMAN

INSTRUCTIONS FOR USING THE VTi 50 VERTICAL TUBE ROTOR

In Beckman Class H and R Preparative Ultracentrifuges



U.S. Pat. No. 4,102,490
U.S. Pat. No. 4,290,550
Japanese U.M. No. 1,469,154

SPECIFICATIONS

Maximum speed	50 000 rpm
Density rating at full speed	1.7 g/mL
Relative Centrifugal Field* at maximum speed	
At r_{max} (86.6 mm) +10	242 000 x *g*
At r_{av} (73.7 mm)	206 000 x *g*
At r_{min} (60.8 mm)	170 000 x *g*
k factor at maximum speed	36
Number of tube cavities	8
Available tubes	see Table 1
Recommended temperature range	see text
Nominal dimensions of largest tube	1 x 3–1/2 in. (25 x 89 mm)
Required spacer and adapter	see Table 1
Nominal rotor capacity	312 mL
Approximate acceleration time to maximum speed (rotor fully loaded, in the L8-80M ultracentrifuge)	20 min
Approximate deceleration time from maximum speed (rotor fully loaded, in the L8-80M ultracentrifuge	14 min
Weight of fully loaded rotor	12 kg (27 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 ($2\pi RPM/60$), and g is the standard acceleration of gravity 9807 mm/s²). After substitution:

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

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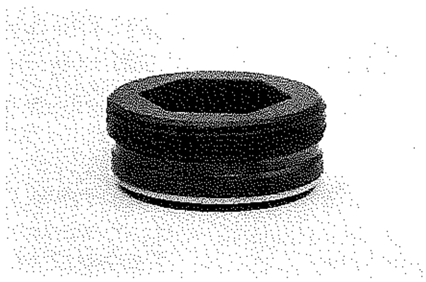
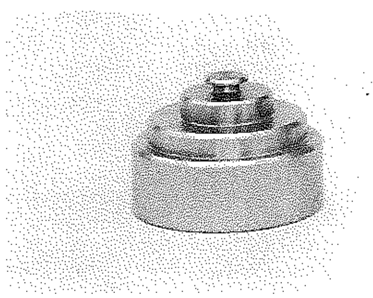
DESCRIPTION

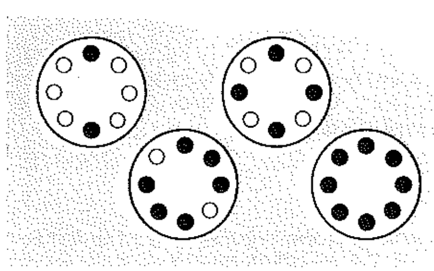
The VTi 50 is a vertical tube rotor, rated for 50 000 rpm. It is designed to centrifuge up to eight Quick-Seal® tubes (see Table 1) in an upright position. Used in class H and R preparative ultracentrifuges, the rotor develops centrifugal forces that can efficiently separate and band DNA, viruses, and subcellular particles. It generates large interband volumes when more than one component is being studied. The rotor is made of titanium, and is finished with black urethane paint. A rotor plug and tube spacer hold each tube in the rotor (Figure 1), and a plug gasket forms a closure around each rotor plug. Rotor plugs are red-anodized aluminum. Spacers are clear-anodized aluminum. Because of the weight of the rotor, there are no drive pins in the rotor drive hole.



Rotor Plug.



Tube Spacer



Tubes arranged symmetrically in rotor.

Figure 1. The VTi 50 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.

A photoelectric detector in the centrifuge monitors the overspeed disk on the bottom of the rotor (see the Supply List) and shuts down the run if speeds exceeding 50 000 rpm are registered. The VTi 50 is warranted for 5000 runs, 10 000 hours of centrifugation, or 5 years, whichever occurs first (see the Warranty).

Table 1. Available Tubes for the VTi 50 Rotor. All may be used at 50 000 rpm.

Shape and Dimensions (in.)	Polyallomer Part No. (Box of 50)	Ultra-Clear Part No. (Box of 50)	Nominal Volume (mL)	Spacer Part No.	Floating Spacer* Part No.	Tube Sealer Rack† Part No.
Bell-top 1 x 1–1/2	343664	344324	15	342417	343448 (two)	344022
Bell-top 1 x 2–1/2	343665	344323	27	342417	343448 (one)	344023
Dome 1 x 3–1/2	342414	344326	39	342417	none	342422

* Floating spacers are made of Noryl, a registered trademark of General Electric.

† Tube Topper rack number is 348124.

OPERATION

NOTE: Specific information about the VTi 50 rotor is given here. Information common to this and other rotors is contained in the Rotors and Tubes Manual, LR-IM, which should be used together with this bulletin for complete rotor and accessory operation.

The VTi 50 rotor uses Quick-Seal tubes (see Table 1). Polyallomer tubes have been tested for use at temperatures between 2 and 25 degrees C and Ultra-Clear™ tubes at temperatures between 4 and 20 degrees C. For use at other temperatures, these tubes should be tested under simulated run conditions beforehand. Ultra-Clear tubes should not be autoclaved or used with solutions of pH greater than 8. See the Rotors and Tubes Manual (LR-IM) for the care, cleaning, sterilization, and chemical resistance of both kinds of Quick-Seal tubes and of rotor components. See the instruction bulletins "How to Use Quick-Seal® Tubes," (IN-163 and IN-180) for filling and sealing the tubes.

The *g*-Max™ system uses a combination of short bell-top Quick-Seal tubes and floating spacers (also referred to as *g*-Max spacers). This system permits centrifuging the shorter 15 and 27 mL tubes (see Table 1) with no reduction in *g* force. For more information on the *g*-Max system, see publication DS-709.

ROTOR PREPARATION AND USE

Before using the rotor, make certain the overspeed disk is properly attached to the bottom of the rotor. If it is missing or has been damaged, do not run the rotor. Replace the disk according to instructions in the

Rotors and Tubes Manual. Be sure that the plug threads in the rotor 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.

NOTE: Do not run an empty rotor. Place filled tubes in at least two opposing cavities. Make sure that these cavities also have the proper floating spacers and/or spacers inserted before installing the rotor plugs.

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. Thus, place one or two floating spacers over the short, bell-top tubes (see Figure 2). Then place a spacer on top of each tube or floating spacer. Inspect the rotor plugs and plug gaskets for damage—the high forces generated in the rotor can cause damaged components to fail. Then insert a rotor plug (gasket-end down) over each spacer and screw it in. (Do not insert plugs into empty cavities.) Tighten each rotor plug to 150 inch-pounds (16.9 N·m), using the torque wrench. *Do this while the rotor is within 50°C of the planned run temperature.* The top surface of each rotor plug should be flush with the surface of the rotor. The rotor is now ready to be placed into an instrument for centrifugation. Consult the appropriate instrument instruction manual for ultracentrifuge operation.

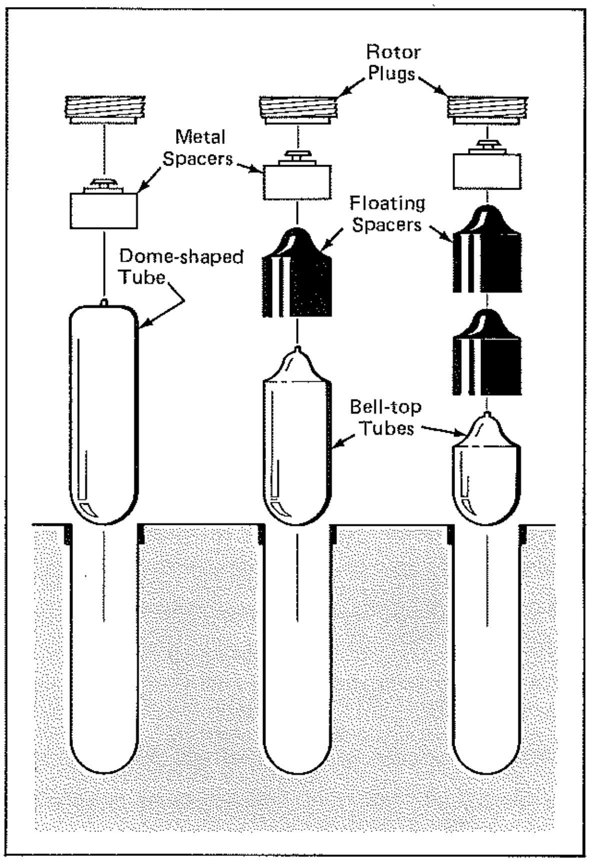


Figure 2. Loading the Rotor with the Correct Combination of Floating Spacer(s) and/or Metal Spacer

Vertical banding of sample and gradient occurs with centrifugation. With deceleration, tube contents reorient back to a horizontal position. For gradient stability when *preformed gradients* are used, the acceleration of the centrifuge drive must be slowed:

- in the L8M series of ultracentrifuges, select a slow acceleration profile.

- in the L8 series of ultracentrifuges, select SLOW ACCEL ON.
- 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 preformed sucrose gradients of less than 5 to 20% (wt/wt), use of the SAA is recommended.
- in L5 and L5B series ultracentrifuges with SAA, turn the SAA on.

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

- in the L8M series of ultracentrifuges, select a slow deceleration profile.
- in the L8 series of ultracentrifuges, select SLOW ACCEL ON (brake will drop out at 400 rpm).
- in L5B series ultracentrifuges, set the BRAKE switch on SLOW or turn the SAA on (brake will drop out between 1500 and 1000 rpm).
- in L5 series ultracentrifuges that either have the SAA installed or that have been internally modified by a Beckman Service Representative, the brake will drop out between 1500 and 1000.
- in L5 series 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 metal spacers and tubes with a hemostat or with the tube removal tool provided. Remove floating spacers with the threaded end of the floating spacer removal tool.

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.

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:

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

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.

Finally, use equation 1 above to calculate the k factor when the column of liquid is such that the operational r_{max} and r_{min} are significantly different than the r_{max} and r_{min} of the rotor (e.g., when using partially filled tubes or adapters). For more information on k factors see the Rotors and Tubes Manual and publication DS-719.

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.

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.

$$\text{reduced maximum speed} = (50\ 000\ \text{rpm}) \sqrt{\frac{1.7\ \text{g/mL}}{\text{density of tube contents}}} \tag{5}$$

Further speed limits must be imposed when CsCl or other self-forming gradient salts are centrifuged, as equation (5) does not predict concentration limits that are required to avoid 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.

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} (86.6 mm)	At r_{av} (73.7 mm)	At r_{min} (60.8 mm)	
50 000	242 000	206 000	170 000	36
45 000	196 000	167 000	138 000	44
40 000	155 000	132 000	109 000	56
35 000	119 000	101 000	83 400	73
30 000	87 300	74 300	61 300	100
20 000	38 800	33 000	27 200	224
10 000	9 700	8 250	6 810	896

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

SELECTING CsCl GRADIENTS

CAUTION

When ionic media are used for density gradient centrifugation, special speed restrictions must be followed to avoid precipitation of the gradient salt.

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, whether you are using 15-, 27-, or 39-mL tubes.

NOTE: The curves in Figure 3 correspond to CsCl dissolved in distilled water. The substitution of buffer containing low levels of other salts for the distilled water will result in equivalent equilibrium density distributions. The presence of high levels of other salts may result in precipitation, however, and should be avoided.

Figure 3b gives the equilibrium gradients that result from centrifugation using the maximum densities allowed by Figure 3a at several run speeds. In general, 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 concentration specified.

TYPICAL EXAMPLES FOR DETERMINING CsCl RUN PARAMETERS

Example A: Knowing homogeneous CsCl solution density (e.g., 1.71 g/mL) and approximate particle densities (e.g., 1.7 and 1.65 g/mL), where will particles band?

1. At 20 degrees C, according to Figure 3a, a solution of 1.71 g/mL should be centrifuged no faster than 40 000 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 (40 000 rpm) and temperature (20 degrees 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. In this case, however, a 40 000 rpm curve is given. Using the horizontal axis, it can be determined that at equilibrium during centrifugation these bands will be 5 mm apart. In the 1 1/2-in. tubes they will be separated by 6.4 mL, and in the 3 1/2-in. tubes by 9.4 mL.

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

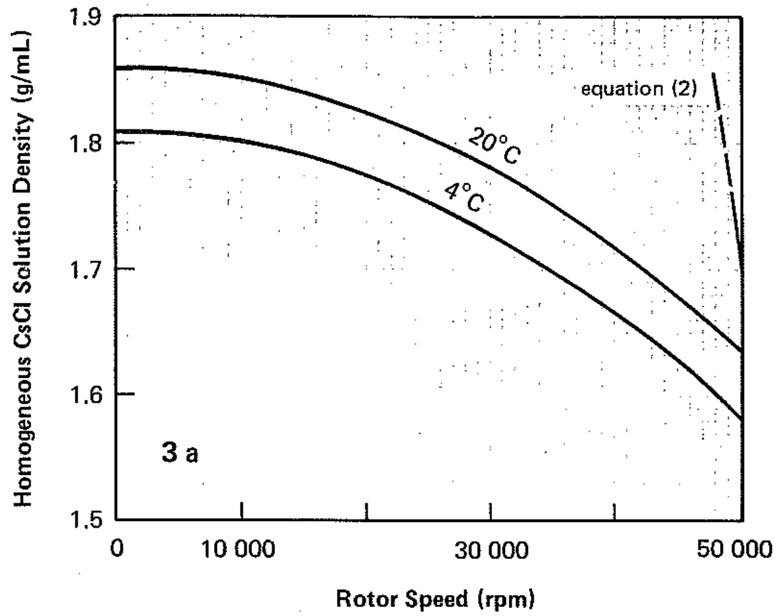
1. In Figure 3b or 3c sketch in a horizontal line corresponding to each particle density.
2. Select the curve that gives the best particle separation at the desired temperature. Particles will band at points across the tube diameter where the sketched lines intersect this curve (lower axis) at equilibrium 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.)

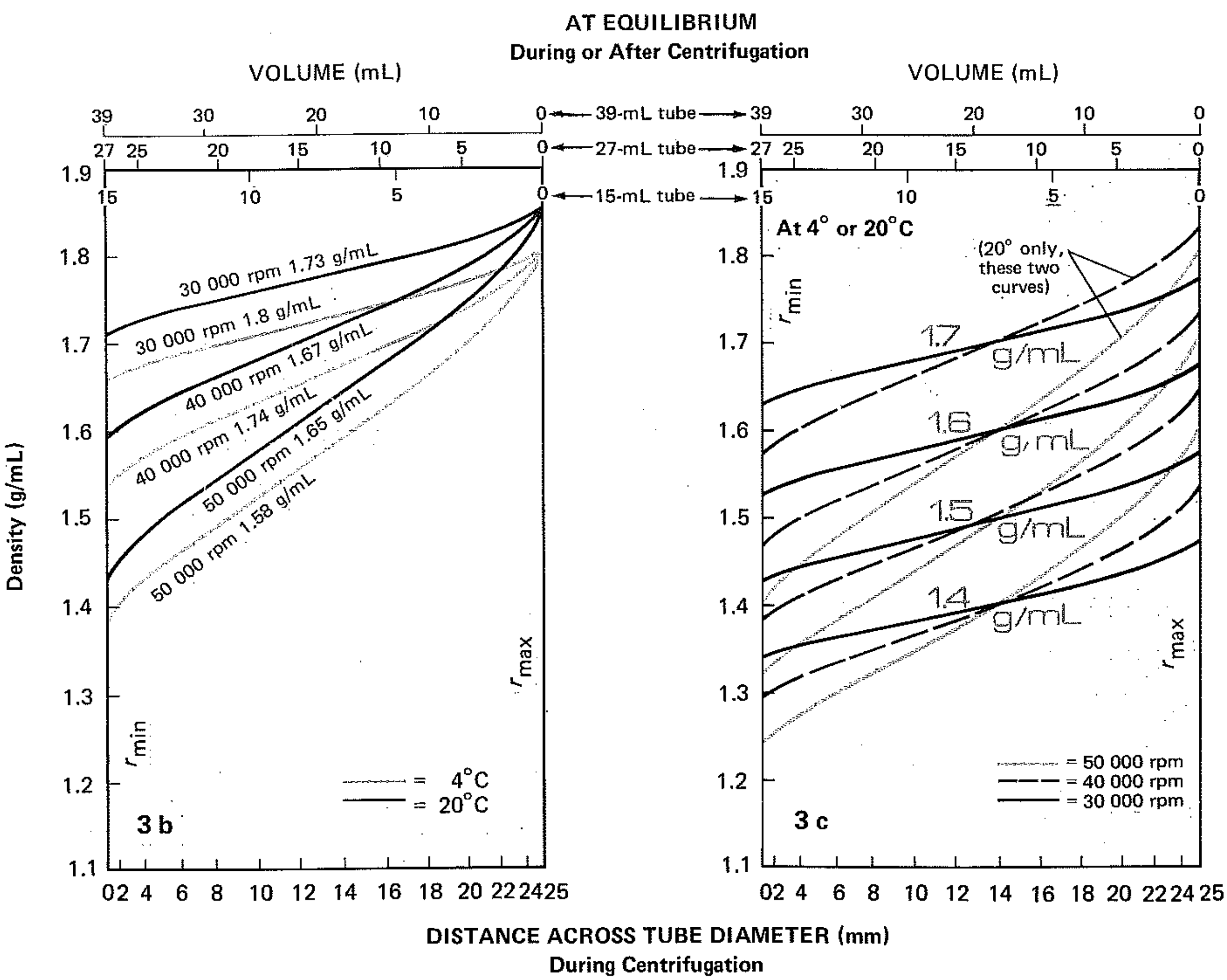
Figure 3. CsCl Precipitation and Equilibrium Curves

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. The dashed line shows the inability of equation (5) to prevent CsCl precipitation.

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.





In this case, the 1.6 g/mL, 30 000 rpm curve in Figure 3c gives the best separation. The curves in Figure 3b are above the density range that would give any separation; and at higher speeds in Figure 3c, the steeper gradients give less distance between particle bands. In Figure 3c the 1.6 g/mL, 30 000 rpm curve intersects the particle densities in such a way that particles band about two millimeters apart at equilibrium during centrifugation (the lower axis).

MAINTENANCE

- Routinely inspect the overspeed disk on the bottom of the rotor. If it is damaged, peel it off and 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 plug gasket a razor blade must be used to cut the gasket from the rotor plug. *Do this carefully so that the plug is not damaged.* The new gasket snaps onto the grooved end of the rotor 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.

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.

SUPPLY LIST

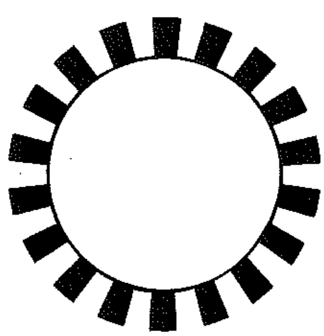
See the Rotors, Tubes, and Accessories catalog (PL-174) for detailed information on reordering supplies. For your convenience, a partial list is given below.

Replacement Rotor Supplies

VTi 50 rotor 340650
Quick-Seal tubes, spacers, floating spacers, racks . . . . . see Table 1
Rotor plug 355587
Plug gasket 340825
Hex plug adapter 355588
600 in.-lb torque wrench 889096
Overspeed disk (50 000 rpm) 330336
Rotor vise assembly 332688
Floating spacer removal tool 338765
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 (tube-sealing accessory, 60 Hz) . . . 348137
Quick-Seal Tube Topper (tube-sealing accessory, 50 Hz) . . . 349647
Tube rack (1-in. diameter, 18 places) 348124
Beckman Fraction Recovery System 343890



Overspeed Disk for the VTi 50 Rotor (36 sectors)