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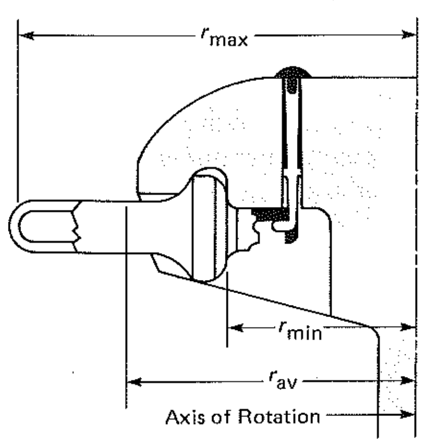
BECKMAN

INSTRUCTIONS FOR USING THE SW 60 Ti ROTOR

In Beckman Class G and H Preparative Ultracentrifuges



U.S. Pat. No. 3, 752, 390;
Canadian Pat. No. 957, 668;
Swiss Pat. No. 550, 611;
Italian Pat. No. 979, 244;
British Pat. No. 1, 415, 600;
German Pat. No. 2, 311, 234;
Japanese Pat. No. 813, 578.

SPECIFICATIONS

Maximum speed	60 000 rpm
Density rating at full speed	1.2 g/mL
Relative Centrifugal Field* at maximum speed	
At r_{max} (120.3 mm)	485 000 x *g*
At r_{av} (91.7 mm)	370 000 x *g*
At r_{min} (63.1 mm)	254 000 x *g*
k factor at maximum speed (full tubes)	45
k′ factors at maximum speed (5-20% sucrose at 5°C)	
When particle density = 1.3 g/mL	126
When particle density = 1.5 g/mL	115
When particle density = 1.7 g/mL	111
Number of buckets	6
Nominal tube dimensions	7/16 x 2 3/8-in. (11 x 60 mm)
Nominal tube capacity	4.4 mL
Nominal rotor capacity	26.4 mL
Available tubes	see Table 1
Approximate acceleration time to maximum speed (rotor fully loaded) in an L8M Ultracentrifuge	3½ min
Approximate deceleration time from maximum speed (rotor fully loaded) in an L8M Ultracentrifuge	2½ min
Weight of fully loaded rotor	4.5 kg (10 lb)
Rotor material	titanium
Conditions requiring speed reductions	see **RUN SPEEDS**

* Relative Centrifugal Field (RCF) is the ratio of the centrifugal acceleration at a specified speed and radius ($r\omega^2$) 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:

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

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DESCRIPTION

The SW 60 Ti swinging bucket rotor, rated for 60 000 rpm, carries six buckets that hold $\frac{7}{16}$ x $2\frac{3}{8}$-inch tubes. Used in Beckman class G and H preparative ultracentrifuges, this rotor can generate high centrifugal forces for the separation of small particles, such as proteins and nucleic acids, using rate-zonal or isopycnic centrifugation techniques. Approximate sample volume per tube is 0.2 mL, with a gradient volume of about 4 mL.

The titanium rotor and buckets are finished with black urethane paint for optimal temperature control. The rotor handle and bucket caps are aluminum and are anodized for corrosion resistance. Bucket and cap assemblies attach to a crossbar on the hanger mechanism in proper position: DO NOT ADJUST THESE SCREWS. O-rings made of Buna N rubber are located between the buckets and the bucket caps to maintain atmospheric pressure inside the buckets during centrifugation. Drive pins in the adapter bottom prevent slippage of the rotor relative to the instrument drive hub during acceleration and deceleration of the rotor. A rotor speed exceeding 60 000 rpm will be detected in class G and H instruments if the 30-sector overspeed disk (shown in the Supply List) is attached to the bottom of the rotor adapter.

The SW 60 Ti rotor is warranted for 1000 runs, 2500 hours of centrifugation, or five years, whichever occurs first, at any speed up to maximum. If, after 1000 runs or 2500 hours of centrifugation, the 5-year warranty period has not expired, the warranty is then extended for an additional 1000 runs, or 2500 hours of centrifugation at any speed up to 90 percent of the maximum, or until the expiration of the 5-year period, whichever occurs first (see the Warranty). Such a rotor is referred to as *derated.*

OPERATION

> **NOTE:** Specific information about the SW 60 Ti 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 in conjunction with this bulletin for complete rotor and accessory operation.

TUBES

Tubes that are available for the SW 60 Ti rotor are detailed in Table 1. Polyallomer and polycarbonate tubes should not be centrifuged below 2°C. These tubes can be centrifuged satisfactorily at elevated temperatures; however, they should be pretested under anticipated run conditions. Ultra-Clear™ tubes have been tested for use at temperatures between 4 and 20°C. For use at other temperatures, pretest these tubes as well under anticipated run conditions.

Table 1. Available Tubes ($\frac{7}{16}$ x $2\frac{3}{8}$ in.) for the SW 60 Ti Rotor

Description	Part Number	Nominal Fill Volume (mL)	Maximum Speed (rpm)
Ultra-Clear	344062	4	60 000
polyallomer	328874	4	60 000
thickwall polyallomer	338787	3	60 000
thickwall polycarbonate	338786	3	60 000

Ultra-Clear and thinwall polyallomer tubes should be filled to within 2 or 3 mm of the top for proper tube support. Thickwall tubes should be half-full to within 2 or 3 mm of the top for centrifugation. Refer to the Rotors and Tubes Manual for cleaning, sterilization, and chemical resistances of tube materials.

ROTOR PREPARATION

NOTE: The instructions to grease bucket O-rings and tighten bucket caps until the numbers are aligned are important! Any misalignment will become progressively worse.

Before using the rotor, be sure the overspeed disk is attached to the adapter bottom. Bucket cap threads should be lightly lubricated with Spinkote™ lubricant,and bucket O-rings should be thinly coated with silicone vacuum grease. Never run a bucket without an O-ring, as it will leak. For runs at temperatures other than room temperature, always refrigerate or warm the rotor beforehand, since titanium is a poor conductor of heat.

Tubes placed in opposing buckets should be filled to the same level with the same density liquid. Two, three, four, or six tubes can be centrifuged, if they are arranged symmetrically on the rotor body (see Figure 1). Hook all buckets to the rotor, whether loaded or empty.

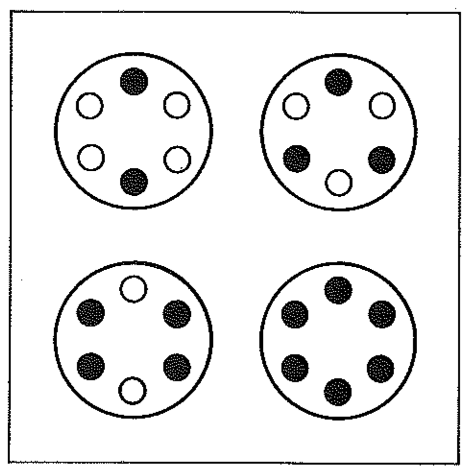


Figure 1. Arranging Tubes Symmetrically in the Rotor Buckets. All buckets must be on the rotor, whether loaded or empty.

Match numbered caps with numbered buckets, and screw the caps into the buckets until there is metal-to-metal contact AND the numbers are aligned. Then attach all buckets to the rotor, whether loaded or empty, by hooking the buckets over the aluminum crossbar in the rotor body (see Figure 2). The crossbar will be visible when the bucket is properly hooked.

CAUTION

The cap hooks can be easily bent, damaged, or fractured if dropped. Handle them carefully. Do not attempt to repair a bent cap. Refer to the maintenance section for more information.

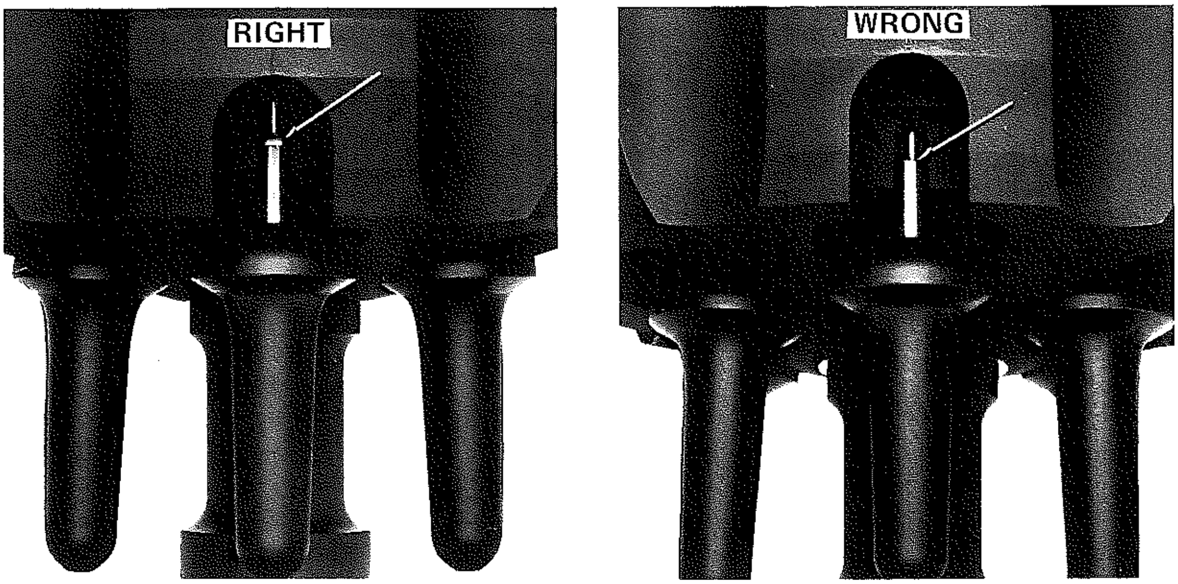


Figure 2. Attaching Buckets to the Rotor Body. The crossbar of the hanger mechanism is visible when the bucket is properly hooked.

Be careful when carrying the loaded rotor and when installing it in the ultracentrifuge. Refer to the Rotors and Tubes Manual for important information on installing the rotor. In instrument Models L2-65B/75B, use the rotor stabilizer in the fourth level (four dots).

NOTE: Consult the appropriate instrument instruction manual for ultracentrifuge operation.

After centrifugation, apply the cap removal tool at the *bottom* of the cap to loosen it (see Figure 3). Failure to use the wrench as shown in the figure can damage the cap hooks.

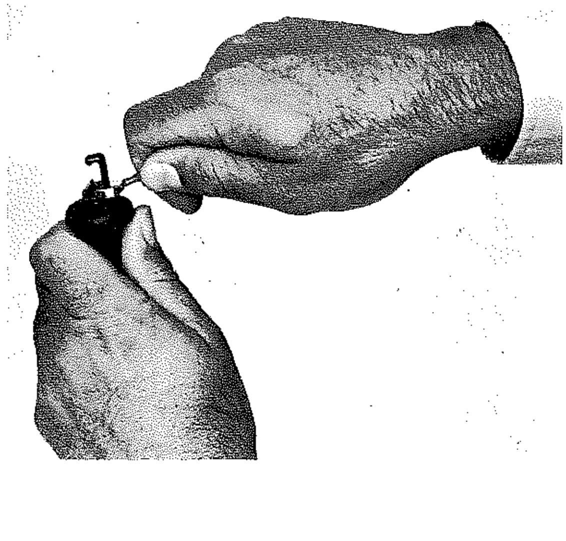


Figure 3. Cap Removal Tool. Use the cap removal tool, part number 001878, to loosen caps after centrifugation.

RUN TIMES

The k factor[1] of the rotor (45 at 60 000 rpm) is a measure of the rotor's relative pelleting efficiency. The k factor can be used in the following equation to estimate the time t (in hours) required to pellet particles of known sedimentation coefficient s (in Svedberg units).

$$t = \frac{k}{s} \qquad (1)$$

Run times can be calculated for runs at less than maximum speed by using the k factors in Table 2 or by adjusting the k factor as shown below.

$$k = 45 \left(\frac{60\,000 \text{ rpm}}{\text{actual run speed}} \right)^2 \qquad (2)$$

Alternatively, k' factors (listed in the specifications for various particle densities) can be used in the above equations to estimate the time required to move a zone of particles of known sedimentation coefficient and density to the bottom of a gradient. If the gradient is isokinetic, or nearly so, use this time to approximate the time required to move the particles a fraction of the distance down the tube length. A more accurate way to determine run times in rate zonal studies is to use the $s\omega^2 t$ charts, available in DS-528 from the Spinco Division of Beckman Instruments. If the values of s and ω^2 are known, and gradients are either 5 to 20% or 10 to 30% (wt/wt) sucrose, the charts enable one to calculate the run time t.

Equilibrium sedimentation run times should not be calculated using k or k' factors. Cesium chloride gradients, for example, generally require about 36 hours of centrifugation for full tubes. Tubes partially filled with gradient solution may require less time for equilibration. See **SELECTING CsCl GRADIENTS.**

RUN SPEEDS

The centrifugal force at a given radius in a rotor is a function of the rotor 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 having been subjected to the same centrifugal force (refer to Table 2).

Table 2. Relative Centrifugal Field and k *Factors at Various Rotor Speeds for the SW 60 Ti Rotor*

Rotor Speed (rpm)	Relative Centrifugal Field (x g)			k Factor
	At r_{max} (120.3 mm)	At r_{av} (91.7 mm)	At r_{min} (63.1 mm)	
30 000	121 000	92 400	63 600	181
35 000	165 000	126 000	86 600	134
40 000	216 000	164 000	113 000	102
45 000	273 000	208 000	143 000	80
50 000	337 000	257 000	177 000	65
54 000	393 000	299 000	206 000	56
55 000	408 000	311 000	214 000	54
60 000	485 000	370 000	254 000	45

[1] 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.

Rotor speeds may not be selected in excess of those provided in Table 1. In addition, rotor speed must be reduced in the following circumstances.

1. After 1000 runs or 2500 hours of centrifugation, permanently derate the maximum rotor speed to 54 000 rpm. Replace the overspeed disk with the disk that limits rotor speed to 54 000 rpm (see the Supply List). Instructions for installing a new disk are given in the Rotors and Tubes Manual.

2. For centrifuging nonprecipitating solutions of densities greater than 1.2 g/mL in this rotor, use the following square-root reduction formula to determine the allowable rotor speed.

$$\text{RPM} = \text{rated speed}^{*} \sqrt{\frac{1.2\ \text{g/mL}}{\rho}} \qquad (3)$$

where ρ = density of tube contents to be run

This speed reduction will protect the rotor from excessive stresses due to the added tube load.

3. When CsCl or other self-forming-gradient salts are centrifuged, the square-root speed reduction formula above usually will not guard against the precipitation of salt crystals. Solid CsCl has a density of 4 g/mL, and if precipitated during centrifugation may cause rotor failure. Precipitation will also alter the density distribution of the gradient, and therefore the sample separation. Figures 4 and 5, together with the description and examples below, show how to reduce rotor speed when using CsCl gradients.

SELECTING CsCl GRADIENTS

NOTE: The curves provided in Figures 4 and 5 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 concentrated at the tube bottom.

Rotor speed is used to control the shape of a CsCl gradient, but to safeguard the rotor, precipitation of CsCl during centrifugation must be prevented. Speed and density combinations that intersect on or below the curves in Figure 4 ensure that CsCl will not precipitate during centrifugation of the SW 60 Ti rotor. Curves are provided at two temperatures: 20°C (black curves) and 4°C (gray curves). Note that for a given initial homogeneous CsCl solution density, the maximum allowable rotor speed increases as the fill volume in the tube decreases from full to ¼-filled.

The curves in Figure 5 show gradient profiles at *equilibrium.* Each curve was generated for a single rotor speed, using the maximum allowable homogeneous densities (one for each fill volume) that avoid precipitation at that speed and temperature.[2] Figure 5 can

*60 000 rpm. If the rotor has been derated, use 54 000 rpm.

[2] The curves in Figure 5 can be generated from step or linear gradients, or from homogeneous solutions.

be used to approximate the banding positions of sample particles. In general, lower speeds provide better particle separation, but may require longer run times to achieve particle equilibrium.

Solutions can be centrifuged faster when a tube is partially filled with gradient and sample. (For centrifuging thinwall tubes partially filled, fill the remainder of the tube with low-density, immiscible liquid, such as mineral oil.) For example, a *full* tube of a 1.61 g/mL homogeneous CsCl solution can be centrifuged no faster than 30 000 rpm at 20°C (from Figure 4). Figure 5 presents the gradient profile, from 1.43 g/mL at the tube meniscus to 1.86 g/mL at the tube bottom. The same solution in a ¾-filled tube can be centrifuged at 20°C at 34 000 rpm. Interpolation of Figure 5 between the 30 000 rpm and 40 000 rpm curves to the ¾-filled level will give the 34 000 rpm curve. The same solution in a ½-filled tube can be centrifuged at 40 000 rpm, and the gradient distribution at equilibrium will be from 1.43 g/mL at the ½-filled level to 1.86 g/mL at the tube bottom. A tube ¼-filled with a 1.61 g/mL solution can be centrifuged at 53 000 rpm.

TYPICAL EXAMPLES FOR DETERMINING CsCl RUN PARAMETERS

Example A: Starting with a homogeneous CsCl solution density (e.g., 1.72 g/mL) and approximate particle densities (e.g., 1.74 and 1.70 g/mL), where will particles band at equilibrium?

1. In Figure 4, find the curve that corresponds to the desired run temperature (20°C) and tube fill volume (½-filled). The maximum allowable rotor speed is determined from the point where this curve intersects the homogeneous CsCl density (30 000 rpm).

2. In Figure 5, sketch a horizontal line corresponding to each particle density.

3. Mark the point where each density intersects the curve corresponding to the maximum speed and selected temperature.

4. Vertical lines drawn through these intersections will show where particles will band along the tube axis.

In this example, particles will band at about 103 and 108 mm from the axis of rotation with about 5 mm of interband separation. (To determine interband volume in milliliters, use $V = \pi r^2 h$, where r is the tube radius in centimeters, and h is the interband separation in centimeters. In this example, the interband volume is about 0.5 mL.)

Example B: Knowing particle densities (e.g., 1.33 and 1.36 g/mL), how do you achieve good particle separation at equilibrium?

1. In Figure 5, sketch a horizontal line corresponding to each particle density.

2. Select the curve at the desired temperature (20°C) and tube volume (full) that gives good separation.

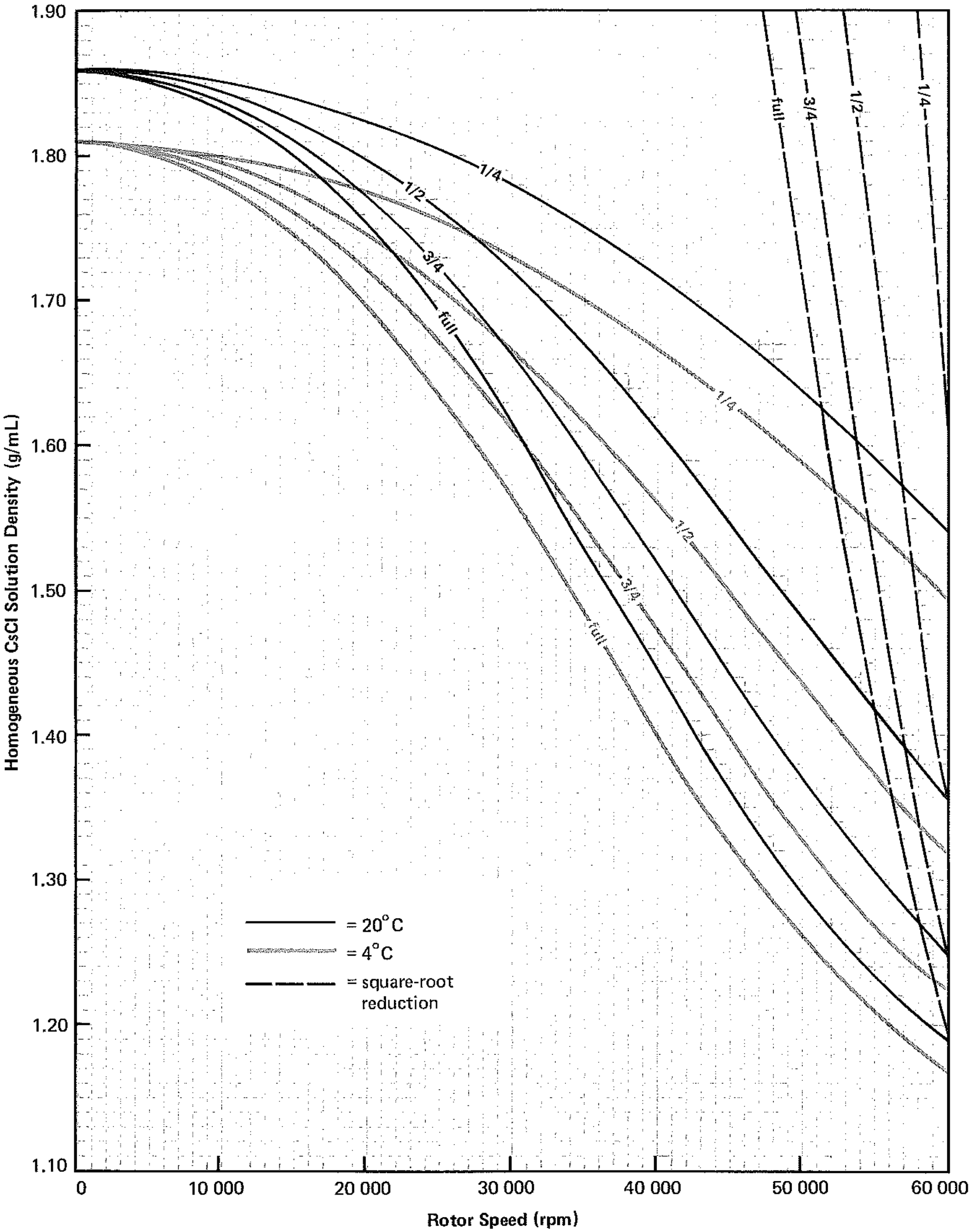
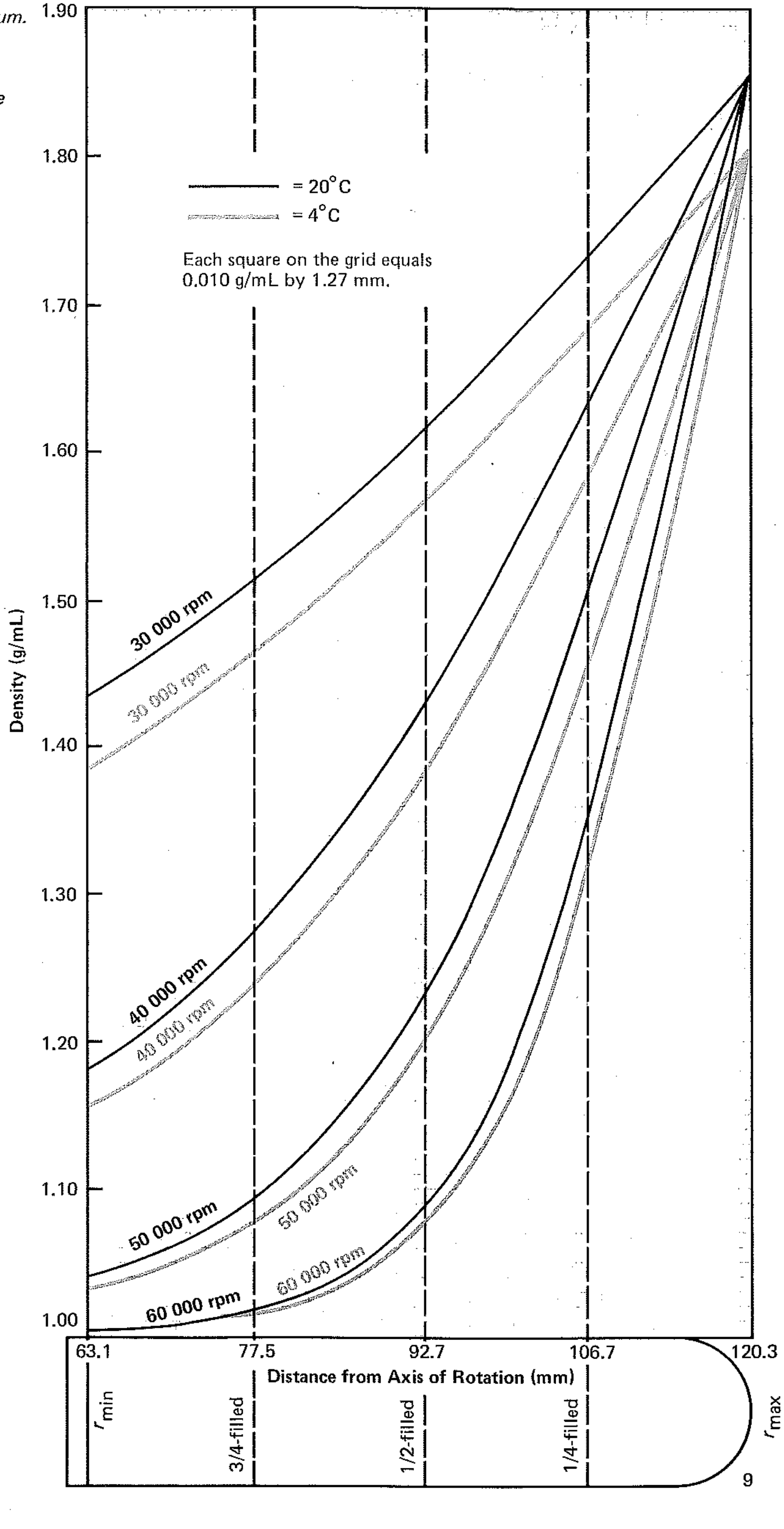


Figure 4. Precipitation Curves for the SW 60 Ti Rotor. Using combinations of rotor speeds and homogeneous CsCl solution densities that intersect on or below the curves ensures that CsCl will not precipitate during centrifugation.

The dashed lines are a representation of equation (3) and are drawn here to show the inability of the square-root reduction formula to prevent CsCl precipitation.

Figure 5. CsCl Gradients at Equilibrium. Centrifugation of homogeneous CsCl solutions at maximum speeds (from Figure 4) results in the gradients presented. Density increases from the top to the bottom of the tube (from 63.1 to 120.3 mm).



3. Note the run speed indicated along the curve (assume 40 000 rpm).

4. From Figure 4, determine the maximum allowable homogeneous CsCl density that corresponds to the selected run parameters (temperature, speed, and fill volume), 1.44 g/mL. These parameters will provide the particle separation selected in Step 2.

In this example, particles will band about 83 and 86 mm from the axis of rotation with about 3 mm of interband separation.

MAINTENANCE

Routinely inspect the overspeed disk. If it is scratched, damaged, or missing, replace it according to instructions in the Rotors and Tubes Manual. Do not use sharp tools on the rotor. Store the rotor in a dry environment (not in the instrument) with the bucket caps removed. Frequently inspect the bucket O-rings for signs of wear. O-rings should be replaced every 6 months, or whenever damaged. Keep the O-rings well lubricated with silicone vacuum grease, and keep the cap threads lubricated with Spinkote lubricant.

INSPECTION

Inspect bucket caps before reuse. To test the caps, attach the empty bucket and cap assembly to the rotor body. Slowly swing it to a horizontal position. An undamaged bucket assembly will move freely. Replace a cap with bent or damaged hooks. DO NOT ATTEMPT TO REPAIR A DAMAGED CAP.

Occasionally inspect the hanger crossbars to make sure they have not loosened or rotated. They should all look the same, and their support screws should all be flush with the top of the rotor. DO NOT TIGHTEN THESE SCREWS. Contact your Beckman Field Service Representative if damage is detected.

CLEANING

If salts or other corrosive materials have been run, or if spillage has occurred, wash the rotor buckets immediately. Do not allow corrosive materials to dry on the rotor. Use a mild detergent solution, such as Solution 555™ diluted 5 or 10 to 1 with water, and brushes that will not scratch the buckets. (The Rotor Cleaning Kit contains two quarts of Solution 555 and two brushes.) Rinse the cleaned buckets thoroughly and air-dry them upside down. Do not immerse the rotor body in water, since the hanger mechanism is difficult to dry and can rust.

STERILIZATION

The rotor buckets, including the O-rings, can be sterilized by autoclaving at 121°C for about one hour. Also, the rotor can be disinfected using 70% ethanol.[3] If the rotor has been contaminated with radioactive or pathogenic materials, appropriate decontamination procedures should be followed. Consult the Rotors and Tubes Manual to select a solvent that will not damage the rotor.

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[3] Flammability hazard. Do not use in or near operating ultracentrifuges.

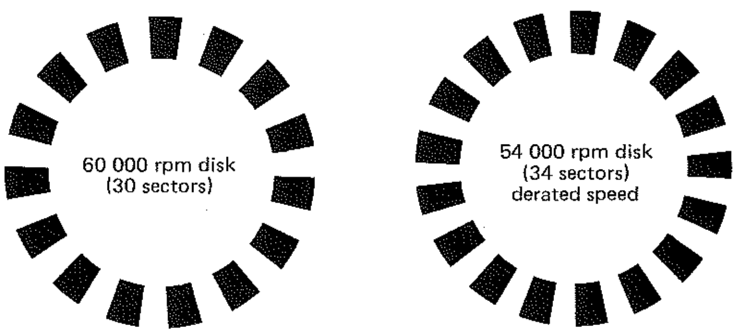
SUPPLY LIST

REPLACEMENT ROTOR SUPPLIES

Bucket assembly, set of six with caps and O-rings	337943
Bucket O-ring	841648
Cap removal tool	001878
Rotor stand	332400
Overspeed disk (60 000 rpm)	331155
Overspeed disk (54 000 rpm)	335459
Bucket holder rack	331313
Tubes	see Table 1

OTHER

Rotor Cleaning Kit	339558
Solution 555	339555
Silicone vacuum grease	335148
Spinkote lubricant	306812



Overspeed Disks for the SW 60 Ti Rotor