L5-TB-069G
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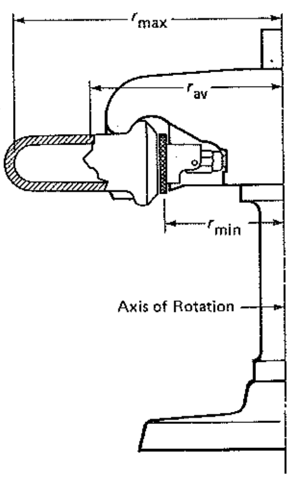
BECKMAN

INSTRUCTIONS FOR USING THE SW 28.1, SW 28, SW 28.1M and SW 28M ROTORS

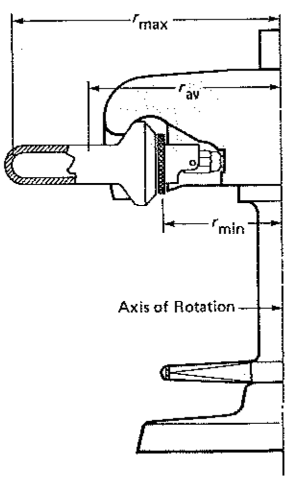
In Beckman Class C, D, F, G, H, and R Preparative Ultracentrifuges

SPECIFICATIONS

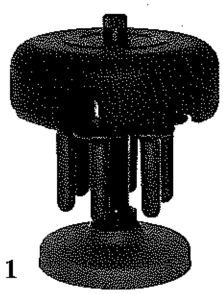
CAUTION
Remove the zonal support band from ultracentrifuges so equipped before operating the SW 28.1 rotor.



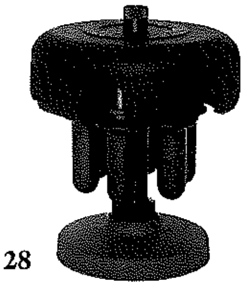
SW 28.1/SW 28



SW 28.1M/SW 28M



SW 28.1



SW 28

	SW 28.1	SW 28
Maximum speed	28 000 rpm	28 000 rpm
Maximum solution density	1.2 g/mL	1.2 g/mL
Relative Centrifugal Field* at maximum speed		
At r_{max} (171.3 mm)	150 000 x g	141 000 x g
At r_{av} (122.1 mm)	107 000 x g	104 000 x g
At r_{min} (72.9 mm)	64 000 x g	66 000 x g
k factor at maximum speed	276	245
k' factors at max. speed (5 to 20% sucrose gradient; 5°C)		
When particle density = 1.3 g/mL	757	680
When particle density = 1.5 g/mL	694	622
When particle density = 1.7 g/mL	668	600
Number of buckets	6	6
Available tubes	See Tables 1 and 2	See Tables 1 and 2
Nominal tube dimensions	$^5/_8$ x 4 in. (16 x 102 mm)	1 x 3 $^1/_2$ in. (25 x 89 mm)
Nominal tube capacity	17 mL	38.5 mL
Nominal rotor capacity	102 mL	231 mL
Approximate acceleration time to maximum speed (rotor fully loaded) in an L8-55M Ultracentrifuge	4 to 5 min	4 to 5 min
Approximate deceleration time from maximum speed (rotor fully loaded) in an L8-55M Ultracentrifuge	4 to 5 min	4 to 5 min
Weight of fully loaded rotor	5.8 kg (12.7 lb)	5.9 kg (13 lb)
Rotor mater	aluminum body; titanium buckets	aluminum body; titanium buckets

*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/s²). After substitution:

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

U.S. Pat. Nos. 4,102,490 and 4,190,195
Canadian Pat. No. 1,120,903

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DESCRIPTION

The SW 28.1 and SW 28 swinging bucket rotors are both rated for 28 000 rpm in Beckman class C, D, F, G, H, and R preparative ultracentrifuges. They are designed to provide a choice of sample volumes in separating subcellular particles and viruses in density gradients. The SW 28.1 rotor uses $^5/_8$ x 4-in. tubes for 0.5 mL of sample with about 16.5 mL of gradient per tube. The SW 28 rotor uses 1 x 3 $^1/_2$-in. tubes for 1 to 2 mL of sample with about 37 mL of gradient per tube. The rotors have a common rotor body with buckets that can be used interchangeably (see Rotor Preparation).

The SW 30.1 and SW 30 rotor buckets can be used on the SW 28.1/SW 28 rotor body as well. *However, the reverse is not true.* The SW 28.1 and SW 28 buckets *cannot* be used on the SW 30.1/SW 30 rotor body. Also, the older SW 27.1 and SW 27 buckets should NOT be used with the SW 28.1/SW 28 rotor body, nor should the SW 28.1 and SW 28 buckets be used on the SW 27.1/SW 27 rotor body.

The SW 28.1 and SW 28 rotor body and handle and the bucket caps are aluminum, anodized for corrosion resistance. The buckets are titanium, finished with red urethane paint. Each bucket and cap assembly attaches to two hooks of the rotor hanger mechanism. Bucket and rotor body positions are numbered for operator convenience. Drive pins in the rotor drive hole prevent slippage of the rotor during acceleration and deceleration.

The SW 28.1 and SW 28 rotors are protected from exceeding 28 000 rpm in instruments with photoelectric overspeed detection systems. An overspeed disk (see the Supply List) is attached to the bottom of the rotor body for this purpose.

> The SW 28.1M and the SW 28M are specially modified versions of the SW 28.1 and SW 28 rotors, to be used in ultracentrifuges that have mechanical overspeed detection systems. Two overspeed cartridges have been added at the base of the rotor body for this purpose (see page 1). *These rotors are otherwise identical to the SW 28.1 and SW 28 rotors.*

The rotors are warranted at maximum speed for 1000 runs, or 2500 hours of centrifugation, or five years, whichever occurs first. 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 maximum. (See the Warranty.)

OPERATION

NOTE: Specific information about the SW 28.1 and SW 28 rotors is given here. Information common to these 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.

TUBES

Tubes available for the SW 28.1 and SW 28 rotors are detailed in Tables 1 and 2. Polyallomer and polycarbonate tubes may be centrifuged at temperatures above 25°C, but they should be pretested under anticipated run conditions. Ultra-Clear™ tubes have been tested for use at temperatures between 4 and 20°C. For centrifugation at other temperatures, pretest these tubes as well. Stainless steel tubes can be centrifuged at any temperature.

Thinwall polyallomer and Ultra-Clear tubes should be filled to within 2 or 3 mm of the top for proper tube support. Thickwall tubes and stainless steel tubes can be centrifuged at any fill level.

Quick-Seal® tubes should be filled almost to the base of the neck. Do not leave a large air space. Too much air can cause the tube to deform, which may disrupt gradient or sample. Consult the Rotors and Tubes Manual or publications IN-163 and IN-181 for detailed information on the care and use of Quick-Seal tubes.

Table 1. Available Open-top Tubes for the SW 28.1 and SW 28 Rotor Buckets

Tube		Nominal Fill Volume (mL)	Maximum Speed (rpm)
Description	Part Number		
SW 28.1			
Ultra-Clear	344061	17	28 000
polyallomer	337986	17	28 000
SW 28			
Ultra-Clear	344058	38.5	28 000
polyallomer	326823	38.5	28 000
thickwall polyallomer	355642	32	28 000
thickwall polycarbonate	355631	32	28 000
stainless steel	301112	38.5	*

*Read the section on Run Speeds before using stainless steel tubes.

The combination of short bell-top Quick-Seal tubes and floating spacers (referred to as the *g*-Max system) is especially useful for centrifuging biohazardous or radioactive samples. The shorter pathlength of these tubes also permits shorter run times. For more information on the *g*-Max system, see publication DS-709.

Table 2. Quick-Seal Tubes for the SW 28.1 and SW 28 Rotors

Tube Description	Dimensions	Part Number	Nominal Fill Volume (mL)	Floating Spacer*	Maximum Speed (rpm)
SW 28.1					
Polyallomer	5/8 x 4 in. 16 x 102 mm	356291	18	355579	28 000
	5/8 x 2 5/8 in. 16 x 67 mm	344622	10		
	5/8 x 2 1/4 in. 16 x 58 mm	344621	8		
	5/8 x 1 3/4 in. 16 x 45 mm	345830	6.3		
	5/8 x 1 1/2 in. 16 x 32 mm	356562	4.2		
SW 28					
Polyallomer	1 x 3 1/4 in. 25 x 83 mm	344623	33	355536	28 000
	1 x 2 1/2 in. 25 x 64 mm	343665	27		
	1 x 1 1/2 in. 25 x 32 mm	343664	15		
Ultra-Clear	1 x 2 1/2 in. 25 x 64 mm	344323	27	355536	28 000
	1 x 1 1/2 in. 25 x 32 mm	344324	15		

*Floating spacers, part of the *g*-Max system of tube support, are made of Noryl, a registered trademark of General Electric.

ROTOR PREPARATION

Before using the rotor, make certain it is equipped with the proper overspeed disk. Refer to the Supply List for part numbers. Be sure the bucket threads are lubricated with Spinkote™ lubricant, and the O-rings are thinly coated with silicone vacuum grease. Never run a bucket without an O-ring, as the bucket will leak. For runs at temperatures other than room temperature, refrigerate or warm the rotor before the run.

Tubes placed opposite each other in the buckets should be filled to the same level with the same density liquid. Be sure to place floating spacers on top of Quick-Seal tubes—these are required for proper tube support (see Figure 1).

Mixed buckets. The two bucket sizes can be mixed if they are arranged symmetrically on the rotor body as shown in Figure 2, using a total of *six buckets* and *six filled tubes.*

Matched buckets. Alternatively two, three, or four tubes can be centrifuged if *six matching buckets* are attached to the rotor and the tubes are arranged symmetrically in the buckets as shown in Figure 2.

Match numbered buckets with numbered caps, and screw caps into the buckets until there is metal to metal contact. Attach six buckets to the rotor, being sure that each bucket is supported by both hooks of the hanger mechanism. Be careful when carrying the loaded rotor and when installing it in the ultracentrifuge, since the buckets can be dislodged. In Model L2-50/65 instruments, use the stabilizer level "25" for the SW 28.1 and SW 28 rotors. In the Model L2-65B/75B, use the top level. Consult the appropriate instrument instruction manual for ultracentrifuge operation.

CAUTION

Remove the zonal support band from ultracentrifuges so equipped before operating these rotors.

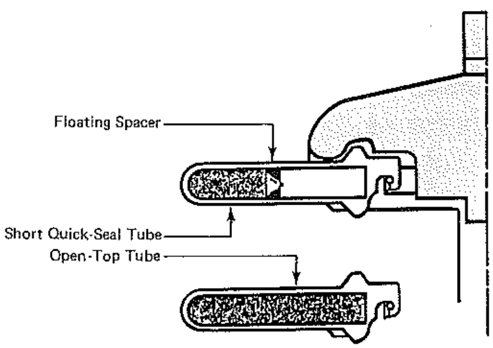

Figure 1. Quick-Seal Tube and Floating Spacer in Swinging Bucket Rotor as Compared to Regular Open-Top Tube

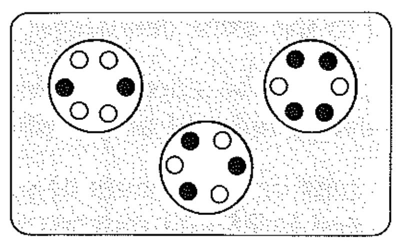

Figure 2. Mix and Match Buckets as Shown Here

RUN TIMES

The k factor of the rotor is a measure of the rotor's pelleting efficiency. 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).

$$t = \frac{k}{s} \tag{1}$$

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

$$k = k_{\text{max speed}} \left(\frac{28\ 000 \text{ rpm}}{\text{actual run speed}} \right)^2 \qquad (2)$$

Alternatively, k' factors (listed in the specifications for various particle densities) can be used in the above equation to estimate the run time required to move a zone of particles of known sedimentation coefficient and density to the bottom of a gradient. The k' factors listed have been calculated for sedimentation through 5 to 20% linear sucrose gradients at 5°C. A more accurate way to estimate run times in rate zonal studies is to use the $s\omega^2t$ 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 run time t.

Equilibrium sedimentation run times should not be calculated using k or k' factors. Cesium chloride gradients, for example, generally require at least 36 hours of centrifugation for full tubes. Tubes partially filled with gradient solution may require less time for particle separation. See Selecting CsCl Gradients below.

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 3).

Rotor speeds may not be selected in excess of 28 000 rpm. In addition, rotor speed must be reduced in any of the following circumstances.

1. After 1000 runs or 2500 hours of centrifugation, permanently derate the rotor speed to 25 000 rpm. At this time replace the 28 000-rpm disk with the 25 000-rpm disk according to the instructions in the Rotors and Tubes Manual (refer to the Supply List for part numbers).

2. For centrifuging nonprecipitating solutions of densities greater than 1.2 g/mL in *plastic tubes*, 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

*28 000 rpm. If the rotor has been derated, use 25 000 rpm.

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

3. For centrifuging *stainless steel tubes*, the speed reduction formula must be modified to account for the added weight of the tube itself. Use the following equation to determine the allowable rotor speed *(for any solution density)*.

$$\text{RPM} = \text{rated speed}^{*}\sqrt{\frac{1.2\ \text{g/mL}}{0.64 + \rho}} \qquad (4)$$

where ρ = density of tube contents

4. When CsCl or other self-forming-gradient salts are centrifuged, the square-root speed reduction formulas 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 3 and 5, together with the description and examples below, show how to reduce rotor speed when using CsCl gradients.

Table 3. 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}	At r_{av}	At r_{min}	
SW 28.1	(171.3 mm)	(122.1 mm)	(72.9 mm)	
28 000	150 000	107 000	64 000	276
25 000	120 000	85 500	51 000	346
21 000	84 600	60 300	36 000	491
20 000	76 700	54 700	32 700	541
15 000	43 200	30 800	18 400	962
SW 28	(161.0 mm)	(118.2 mm)	(75.3 mm)	
28 000	141 000	104 000	66 100	246
25 000	113 000	82 700	52 700	308
21 000	79 500	58 400	37 200	437
20 000	72 100	53 000	33 700	481
15 000	40 600	29 800	19 000	856

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

*28 000 rpm. If the rotor has been derated, use 25 000 rpm.

SELECTING CsCl GRADIENTS

Rotor speed is used to control the slope of a CsCl density gradient. However, maximum rotor speed must be reduced to prevent precipitation of CsCl during centrifugation. Speed and density combinations that intersect on or below the curves in Figures 3 and 5 ensure that CsCl will not precipitate during centrifugation of the SW 28.1 and SW 28 rotors, respectively. Curves are provided at two temperatures: 20°C (black curves) and 4°C (gray curves). The curves in Figures 4 and 6 show gradient profiles at equilibrium for the SW 28.1 and SW 28 rotors, respectively. Each curve was generated for a single rotor speed using the maximum homogeneous CsCl density (from Figures 3 and 5) that avoids precipitation at that speed and temperature.[1] Figures 4 and 6 can be used to approximate the banding positions of sample particles. In general, lower run speeds provide better resolution, but may require longer run times to reach particle equilibration. Curves not shown in the figures may be interpolated. Note that the 28 000 rpm curves are identical for 20 and 4°C.

> **NOTE**: The curves in Figures 3, 4, 5, and 6 are for solutions of CsCl salt only. If other salts are present in significant concentrations, the overall Cscl concentration or rotor speed must be reduced. This prevents precipitation of salts concentrated at the tube bottom.

The following discussion of the CsCl curves uses Figures 3 and 4 for the SW 28.1 rotor as the example.

Tubes partially filled with gradient and sample can be centrifuged faster and for shorter run times. (For partially filled thinwall tubes, fill the remainder of the tubes with a low-density, immiscible liquid, such as mineral oil.[2]) For example, a full tube of a 1.67 g/mL CsCl solution can be centrifuged no faster than 15 000 rpm at 4°C (from Figure 3). The same solution in a 3/4-filled tube can be centrifuged at 17 000 rpm, and a 1/2-filled tube at 20 000 rpm. A tube 1/4-filled with a 1.67 g/mL solution can be centrifuged at 26 000 rpm at 4°C in the SW 28.1 rotor. The gradient profiles in partially filled tubes centrifuged at maximum speeds and CsCl densities are shown in Figure 4.

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[1] The equilibrium curves in Figures 4 and 6 will result from homogeneous solutions, but can be more rapidly generated from step or linear gradients, as long as the total CsCl concentration in solution is equal to the homogeneous solutions from the curves in Figures 3 and 5.

[2] Do not use an oil overlay in Ultra-Clear tubes.

TYPICAL EXAMPLES FOR DETERMINING RUN PARAMETERS

Example A: Starting with a homogeneous CsCl solution density (e.g., 1.33 g/mL) and approximate particle densities (e.g., 1.30 and 1.35 g/mL viruses), where will particles band?

1. In Figure 3 find the curve that corresponds to the desired run temperature (20°C) and tube fill volume (1/2-filled). The maximum allowable rotor speed is determined from the point where this curve intersects the homogeneous CsCl density (28 000 rpm).
2. In Figure 4, sketch in a horizontal line corresponding to each particle density.
3. Mark the point where each particle density intersects the curve corresponding to the allowable speed and temperature.
4. Vertical lines drawn from these intersections will show where particles will band at equilibrium.

In this example, particles will band at about 145 and 151 mm from the axis of rotation with about 6 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 there will be about 1.2 mL of interband volume.)

Example B: Knowing particle densities (e.g., 1.55 and 1.50 g/mL) how do you achieve good particle 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., 4°C) and tube volume (e.g., full) that gives good particle separation.
3. Note the speed indicated along the selected curve (20 000 rpm).
4. From Figure 3, determine the maximum allowable homogeneous CsCl density that corresponds to the selected run parameters (temperature, rotor speed, and fill volume), i.e., 1.56 g/mL. These parameters will provide the particle separation selected in Step 2.

In this example, particles will band at about 110 and 122 mm from the axis of rotation with about 12 mm of interband separation. If a 3/4-filled tube is used instead of a full tube, at 20 000 rpm and 4°C, Figure 3 shows that a 1.61 g/mL homogeneous CsCl solution should be used to generate the gradient profile, and particles will band at the same positions but nearer the top of the gradient.

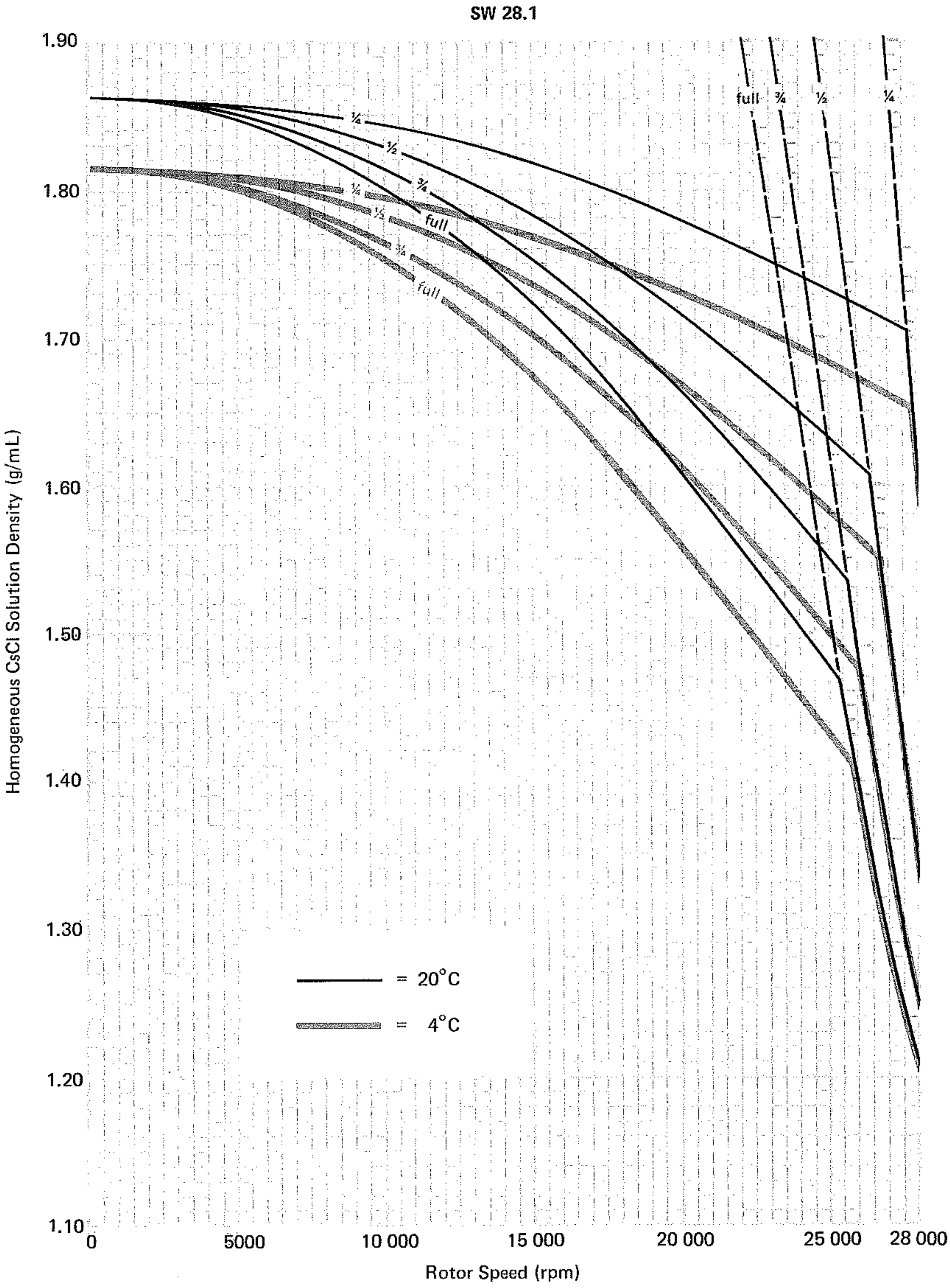

Figure 3. Precipitation Curves for the SW 28.1 Rotor. Using speed and density combinations that intersect on or below the solid curves ensures that CsCl will not precipitate during centrifugation. Tube fill volumes are indicated on the curves. The dashed lines are a respresentation of Equation (3) and are shown here to illustrate the inability of that equation to prevent CsCl precipitation.

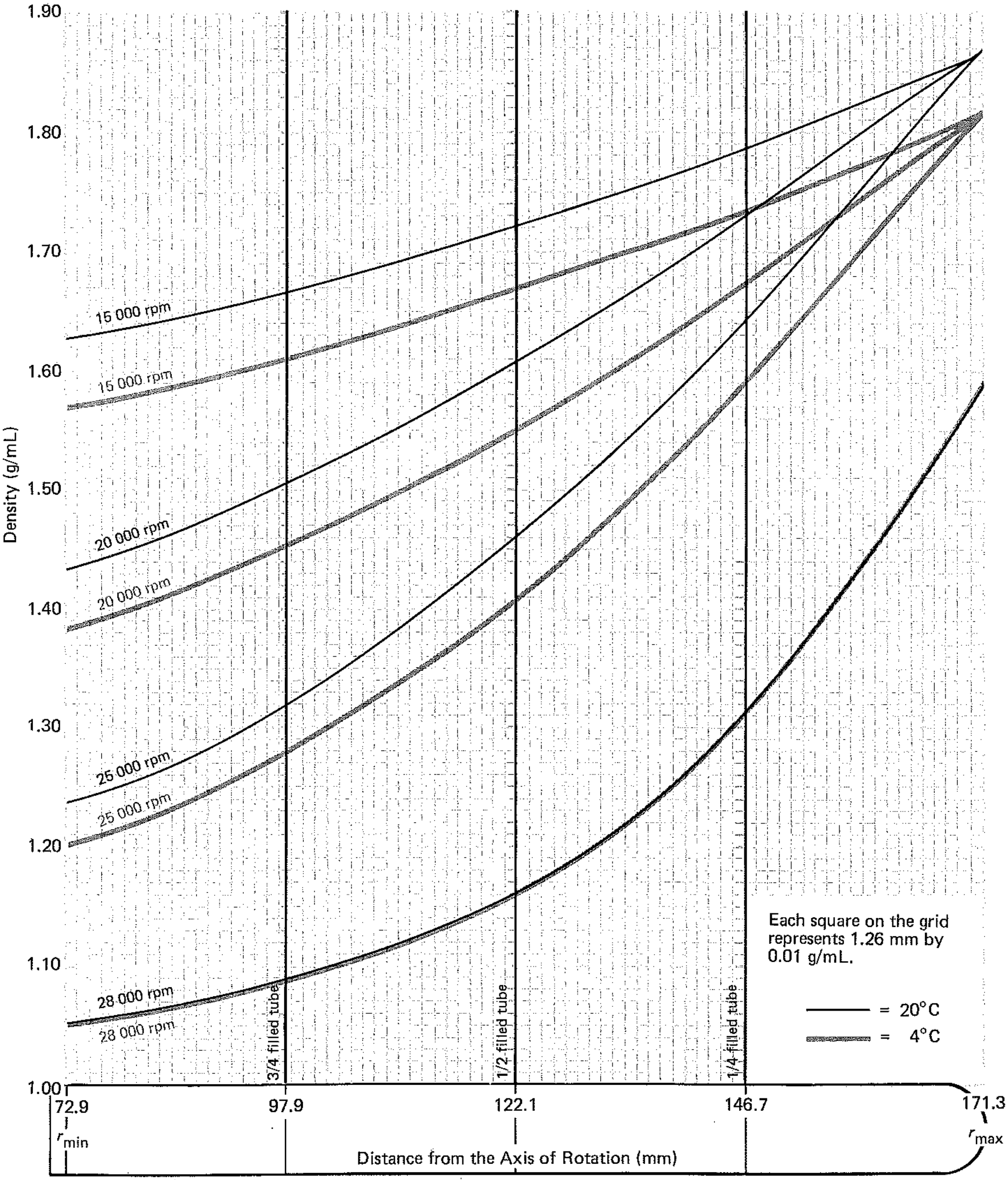

Figure 4. CsCl Gradients at Equilibrium for the SW 28.1 Rotor. Centrifugation of homogeneous CsCl solutions at maximum allowable speeds (from Figure 3) results in the gradients presented.

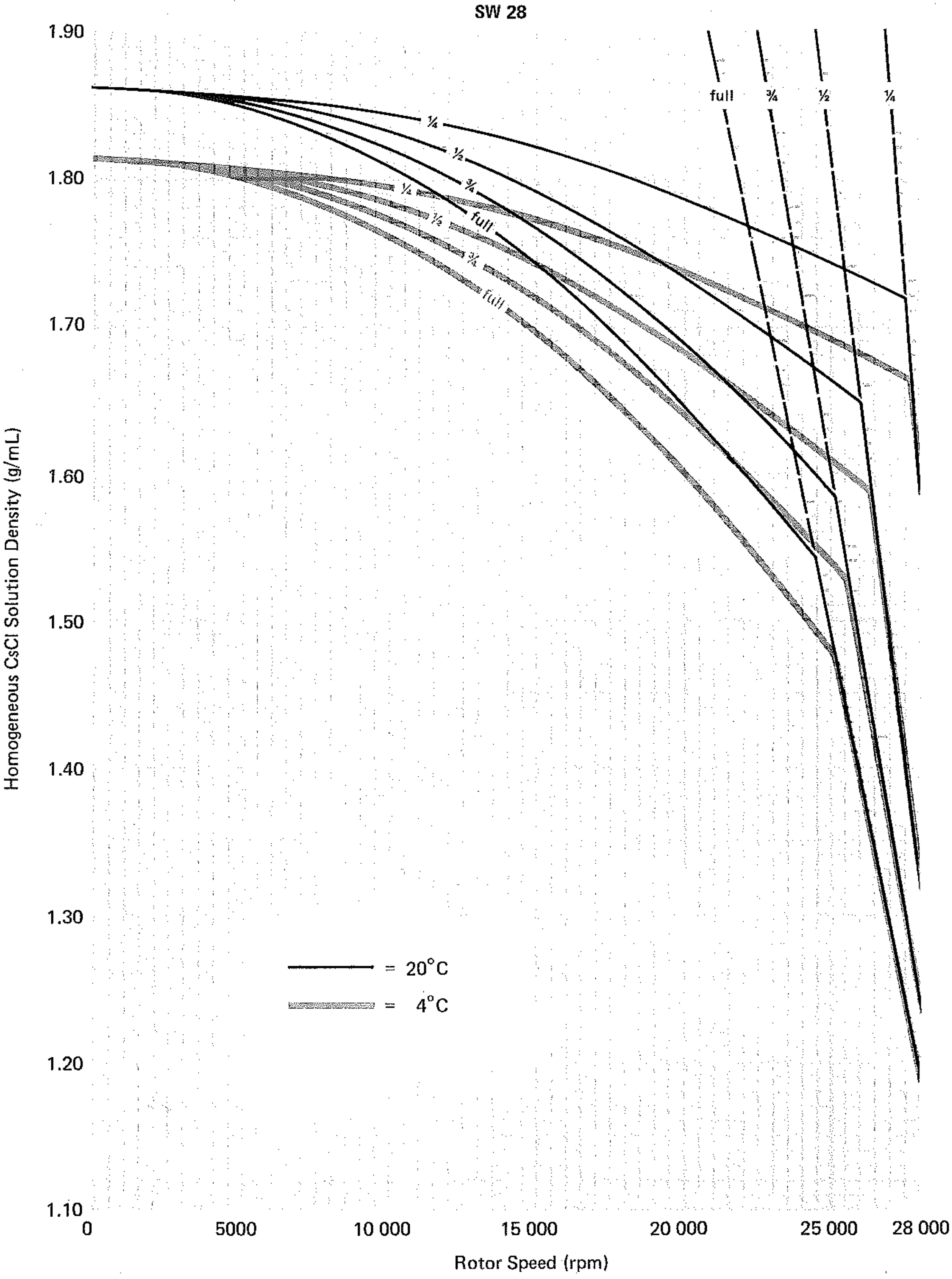

Figure 5. Precipitation Curves for the SW 28 Rotor. Using speed and density combinations that intersect on or below the solid curves ensure that CsCl will not precipitate during centrifugation. Tube fill volumes are indicated on the curves. The dashed lines are a representation of Equation (3) and are shown here to illustrate the inability of that equation to prevent CsCl precipitation.

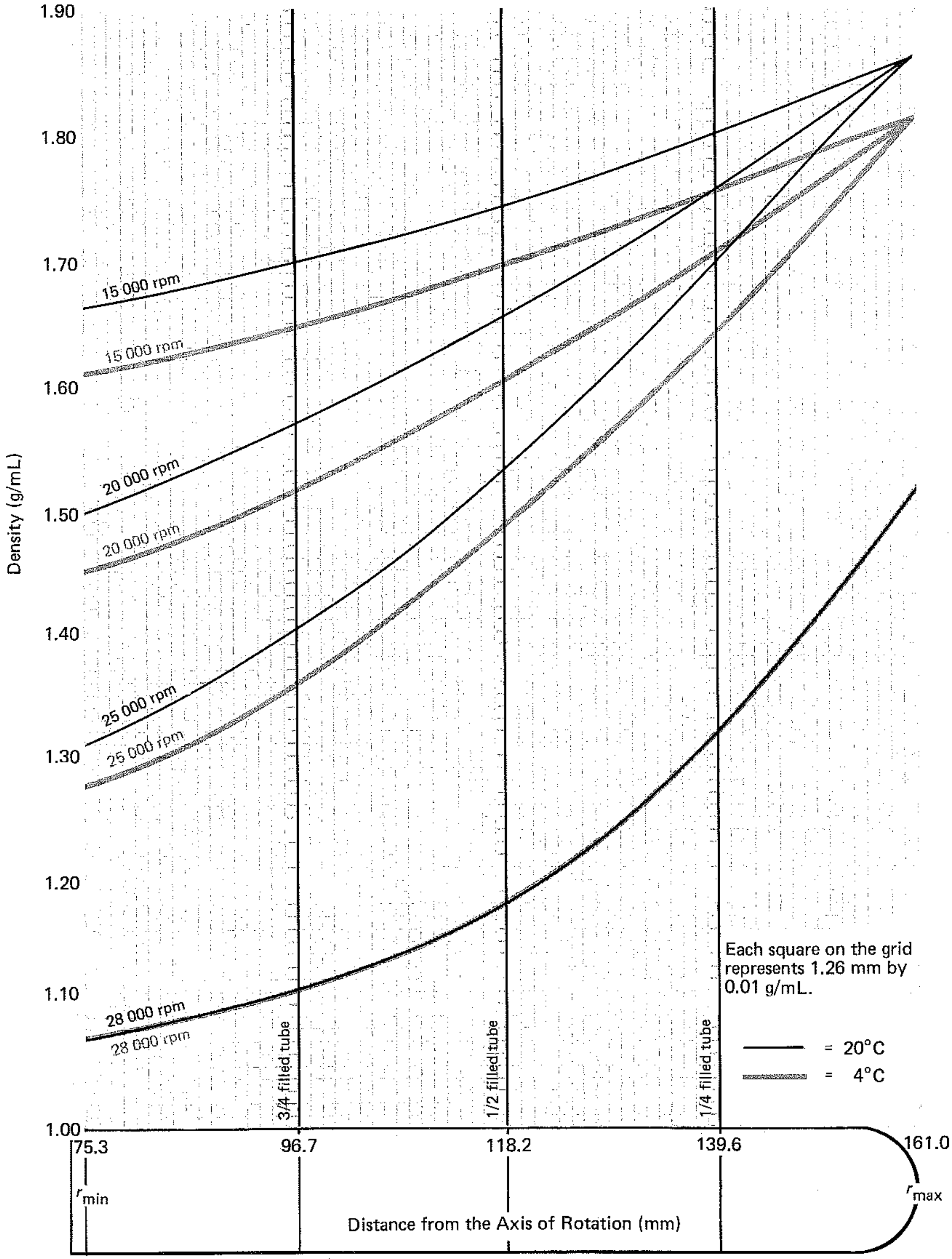

Figure 6. CsCl Gradients at Equilibrium for the SW 28 Rotor. Centrifugation of homogeneous CsCl solutions at maximum allowable speeds (from Figure 5) results in the gradients presented.

MAINTENANCE

Routinely inspect the overspeed disk. If it is damaged or missing, replace it. Refer to the Rotors and Tubes Manual for replacement instructions.

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 check the bucket O-rings for signs of wear. O-rings should be replaced every 6 months, or whenever damaged. Keep the O-rings coated with silicone vacuum grease, and keep the cap threads lubricated with Spinkote lubricant. Refer to the Rotors and Tubes Manual for the chemical resistances of rotor and tube materials. Contact your Beckman Representative for information about the rotor repair center and the Field Rotor Inspection Program.

CLEANING AND STERILIZATION

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.

The rotor body and 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. Refer to the Rotors and Tubes Manual to select a solvent that will not damage the rotor.

DECONTAMINATION

A rotor (and/or accessories) contaminated with radioactive material should be decontaminated using a solution that will not damage its anodized surface. Beckman has tested a number of solutions and found two which do not harm anodized aluminum: RAD-CON (Nuclear Associates, Carle Place, New York 11514) and RADIACWASH (Atomic Products Corp., Center Moriches, New York 11934). Beckman does not, however, warrant the performance of these products with respect to their effect on the rotors (and/or accessories) or their ability to decontaminate these parts.

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

SUPPLY LIST

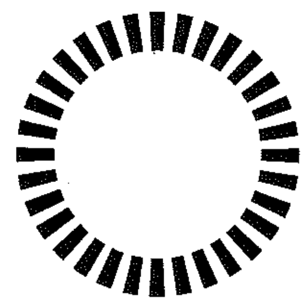
See the Rotors, Tubes, and Accessories catalog (PL-174) for detailed information on reordering supplies. For your convenience, a partial list is given below.

Replacement Rotor Supplies

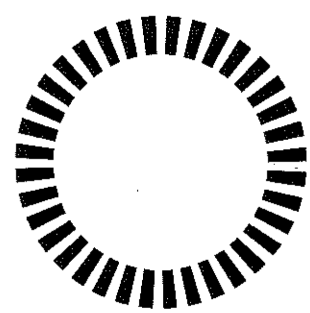
SW 28.1 rotor	342216
SW 28.1M rotor (with mechanical overspeed device)	358106
SW 28.1 buckets (set of 6, with caps and O-rings)	342212
O-rings	815472
SW 28 rotor	342207
SW 28M rotor (with mechanical overspeed device)	358105
SW 28 buckets (set of 6, with caps and O-rings)	342217
O-rings	812715
Tubes	see Tables 1 and 2
Overspeed disk (28 000 rpm)	342211
Overspeed disk (25 000 rpm)	330333
Rotor stand	332400
Bucket holder rack	331186

Other

Tube Topper™ kit (60 Hz)	348137
Tube Topper kit (50 Hz)	349647
Tube racks for the Tube Topper	
for 5/8-in. diameter tubes	348123
for 1-in. diameter tubes	348124
Tube sealing kit (with 60-Hz, 120V sealer)	342429
Tube sealing kit (with 50-Hz, 220V sealer)	342424
Tube racks for the tube sealing kit	
for 5/8 x 1 1/2-in. tubes	356892
for 5/8 x 1 3/4-in. tubes	344641[4]
for 5/8 x 2 1/4-in. tubes	344640
for 5/8 x 2 5/8-in. tubes	344641
for 5/8 x 4-in. tubes	356893
for 1 x 1 1/2-in. tubes	344022
for 1 x 2 1/2-in. tubes	344023
for 1 x 3 1/4-in. tubes	344642



64–sector 28 000 rpm



72–sector 25 000 rpm (derated speed)

[4]Insert the 345828 spacer upside down in this rack to accommodate this shorter tube.