Titanium Centerpieces and a Modified Temperature Control System for the Spinco Analytical Ultracentrifuge

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Received July 27, 1967

Analytical ultracentrifugation of nucleic acid in the concentration range of 5 to 40 μg/ml has been plagued with two serious experimental problems:

1. The centerpieces commercially available from Spinco Division, Beckman Instruments, have serious chemical and mechanical limitations. For example, aluminum and aluminum-filled Epon centerpieces inevitably result in a contamination of the solution with aluminum ions. This is especially true for extreme pH or for solutions with high salt concentration (1-3). The Kel-F centerpieces flow at moderate temperatures (60-70°C) (4, 5) and even at room temperature become deformed after repeated use. There have been some recent indications that the carbon filling in carbon-filled Epon centerpieces leads to chemical problems with dye solutions (6).

2. The second problem is related to convection in the cell. Hearst and Vinograd (7) were unable to obtain a linear extrapolation to zero concentration for the sedimentation coefficient of T4 bacteriophage DNA, and the $s_{\text{in,w}}$ value they obtained is now known to be erroneous. Eigner, Schildkraut, and Doty (8) observed boundary instability at rotor speeds below 15,000 rpm, and Freifelder and Davison (9) reported poor reproducibility of sedimentation coefficients for T7 bacteriophage DNA in dilute salt solution; these last authors were forced to use much higher salt concentrations to obtain reliable data.

The difficulty is believed to arise from convection caused by thermal gradients in the ultracentrifuge cells by the standard ultracentrifuge temperature-control system and also from the poor heat conductivity of some of the materials used in the construction of the centerpieces for

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the ultracentrifuge cells. Extreme sensitivity to small thermal fluctuations is expected in sedimenting systems in which the stabilizing density gradients produced by high salt or DNA concentrations are not present. The problem of boundary instability caused by thermal gradients is aggravated at higher temperatures.

This report describes titanium centerpieces we have used in this laboratory for approximately two years. The titanium is resistant to acid, base, high salt content, and high temperature and does not deform from extended use. In addition, design modifications of the temperature-control system of the analytical ultracentrifuge are described which reduce convective disturbances in the cell. As a result of these two improvements, boundary sedimentation velocity experiments on dilute solutions of DNA are being routinely performed at temperatures as high as 50°C in solvents with ionic strength less than 0.3.

TITANIUM CENTERPIECES AND GASKETS

The dimensions used in the construction of both single- and double-sector centerpieces are those of the standard 12 mm aluminum Spinco centerpieces, with 4° sectors for single-sector and 2° sectors for double-sector centerpieces (one 6 mm, 3 mm, and 1.5 mm centerpiece of each type was also made). Two views of the double-sector centerpieces are shown in Figures 1 and 2. The double-sector centerpieces require a groove between sectors (Figs. 1 and 2) to form a seal with the gaskets and prevent transfer of liquid between compartment.

![Double-sector titanium centerpiece](image-url)
In the case of the single-sector type, the metal workers were able to drill out the rough outlines of the sector and smooth the walls with a specially made broach. Construction of the double-sector centerpieces is considerably more difficult. They were constructed at the Lawrence Radiation Laboratory machine shops using a recently developed electric discharge machining process (10): A brass tool is constructed, consisting of two prongs of the required sector shape. The tool and the titanium workpiece are immersed in a liquid dielectric. The tool is brought very close to the workpiece, and a very high pulsed current passed. The tool then melts the titanium as it passes through the workpiece.

The only disadvantage associated with the use of titanium centerpieces is the added weight over commercial centerpiece materials. The maximum rated speed of rotors must be reduced by 10% when using the titanium centerpieces. The heaviest counterbalance weights available for insertion into standard Spinco counterbalances when the titanium centerpieces were first constructed were found to be insufficient for the achievement of balance between a centrifuge cell containing a titanium centerpiece and the counterbalance. Recently, however, Spinco has made available counterbalance weights of tungsten alloy. Although the heaviest of these weighs the same as the heaviest of the older brass weights, the higher density of tungsten allows the new weights to be shorter. Hence, a small \( \frac{3}{2} \) or 1 gm weight may be inserted into the same counterbalance with the heaviest (14 gm) tungsten alloy weight, and the combination is sufficient to balance a cell with a titanium centerpiece, even if filled with liquid of density as high as 2.0 gm/cc. Balance can be achieved regardless whether single- or double-sector centerpieces are used.

The single-sector centerpieces require gaskets between the centerpiece and the cell windows, as do all metal centerpieces. A gasket punch was constructed to make the gaskets, and worked so well that use of this type of gasket cutter is strongly recommended for all centerpiece gasket

![Fig. 2. Double-sector titanium centerpiece, top view.](image-url)
The punch, illustrated in Figure 3, is made from 7/8" drill rod, which is hardened afterward. The gaskets are punched by placing the gasket material on a hardwood block, placing the cutting end of the punch on the material, and striking the end of the punch a sharp blow with a soft-headed hammer. This proved to be much easier than cutting gaskets with the commercially available device.

The double-sector titanium centerpieces require gaskets with a strip across the center to prevent flow of liquid from one compartment to the other. Punches for making these gaskets were constructed from 7/8" drill rod or tool steel (Fig. 4). The piece across the center was constructed separately of tool steel (without the V-shaped cut) and inserted into a notch cut for it in the body of the punch. The V-shaped cutting edge was then milled out of the crosspiece. Gaskets are punched exactly as with the single-sector gasket punch.

For ultracentrifuge runs below about 40°C, the red polyethylene supplied by Spinco as gasket material may be used. At higher temperatures, polyethylene softens and flows under the centrifugal pressure applied to the cell, and leaks may result. The use of 0.005" Teflon sheet as gasket material at the higher temperatures proved satisfactory. The material
is easy to cut and does not soften at temperatures attainable in the ultracentrifuge.

A test of the Teflon gasket material has been made with a double-sector titanium centerpiece with one compartment filled with water and the other empty. At speeds as high as 50,000 rpm, no leakage between compartments or into the rotor chamber was detected. The double-sector gaskets of red polyethylene are similarly leakproof up to about 50,000 rpm.

MODIFIED HEATING- AND TEMPERATURE-CONTROL SYSTEM

The commercial Spinco model E analytical ultracentrifuge is equipped with the standard RTIC (Rotor Temperature Indicator and Control) unit (11) and can be provided with a high-temperature package (12) for operation above 40°C. The RTIC system maintains the rotor temperature constant to within 0.1°C by means of a temperature-sensing bridge and

![Sketch of gasket punch for double-sector centerpiece gaskets. Materials are ⅛" drill rod or tool steel with tool steel for crospiece.](image)

Fig. 4. Gasket punch for double-sector centerpiece gaskets. Materials are ⅛" drill rod or tool steel with tool steel for crospiece.
relay system, which switch the current to a small heating coil at the bottom of the rotor chamber on and off to maintain the temperature near that desired. For high-temperature operation (above 40°C), a constant, experimentally determined voltage is applied to a larger heating coil (HT coil) wound about the inside of the chamber. The voltage applied to the HT coil is sufficient to maintain the rotor temperature slightly below that desired; the temperature is then controlled by the RTIC unit as described above. The RTIC-HT system is unsatisfactory for boundary sedimentation runs at low DNA and salt concentrations.

The temperature-control system has been revised to eliminate the following objections to the commercial RTIC-HT apparatus: (1) The relative locations of the RTIC temperature control coil and the refrigeration coil (Fig. 5) is such that heat flow from the hot coil through the rotor to the cold refrigeration coil might occur as suggested by the solid curved arrows in Figure 5, creating thermal gradients and causing convection. (2) The height of the commercial HT coil did not exceed the vertical dimensions of the rotors used (Fig. 5) so that the heat flow from rotor through HT coil to refrigeration coil could be as shown by the broken curved arrows, again resulting in convection. Heat flow perpendicular to the axis of rotation may or may not cause convection, depending on the magnitude and direction of the thermal gradient produced; heat flow as in 1 or 2 above will always cause convection if the thermal gradients are sufficiently large.

The design of the modified system thus includes an enlarged HT coil, which exceeds the vertical dimensions of all the rotors and is about the same height as the refrigeration coil. The on- and off-control voltage, instead of being applied to the RTIC coil at the chamber bottom, is

Fig. 5. Side view of rotor in rotor chamber showing positions of RTIC, HT, and refrigeration coils, the latter two coils being viewed in cross-section only. The broken and solid curved arrows indicate possible lines of heat flow with the commercial RTIC-HT system as discussed in the text.
applied to the enlarged HT coil as an increment to a constant voltage. As with the commercial system, the constant voltage is experimentally determined to be that which will maintain the rotor slightly below the temperature desired.

The circuit diagram for the modified temperature control system is shown in Figure 6, with the required parts listed in the legend to the figure. The 6 V on-off output from the RTIC unit, which normally heats the RTIC temperature coil C₂, may be used either to trip a relay (R₁) which cuts in the incremental voltage to the large HT coil (C₁) or for its original purpose, depending upon the position of switch S₁. When relay R₂ is tripped on, an additional voltage of from 0 to 5 V is applied to the primary of transformer T₂ through transformer T₃, depending upon the setting of the small variable autotransformer T₄. As the total resistance of coil C₁ is only about ½ ohm, the 12 V secondary of transformer T₂ allows currents of from 0 to about 24 A to be passed through C₁, depending on the setting of the large variable autotransformer T₁. Such currents are more than sufficient; a rotor temperature of 70°C could be maintained with the autotransformer T₁ set at only about 55 V.

The detailed construction of the HT coil is illustrated in Figure 7.

![Circuit diagram of modified temperature-control system](image)
About 9 ft of 0.128" diameter Nichrome wire (Techalloy “A” No. 8, E. A. Wilcox and Co., Los Angeles, Calif.) is bent into the shape illustrated in Figure 7 (in this laboratory, the number of U-bends is such that the wire would be crossed ten times in going from top to bottom of the figure). If the structure represented in Figure 7 is now bent into a circle so that A is adjacent to A', B adjacent to B', etc., the desired heating coil, corresponding to a helical coil of ten turns, is produced.

![Figure 7](image)

**Fig. 7.** Two-dimensional layout of modified HT coil. Actual coil is bent into a circle, so that A and A', B and B', etc. are adjacent.

The commercial HT system includes a silvered radiation can (reflector) which fits concentrically into the rotor chamber, between the refrigeration coil and the HT coil. The original HT coil was mounted to this reflector, and it has been used for the same purpose with the enlarged HT coil. Six notched supports are cut from 3/8" sheet Teflon and bolted vertically to the inside of the reflector. The HT coil has been mounted in the notches of the supports. The same electrical connections as for the original HT coil were used.

The Teflon supports provide the necessary insulation of the HT coil from the reflector and are able to withstand the highest temperatures required. The highest temperatures occur when the autotransformer T₁ (Fig. 6) is turned to approximately 80 V in order to volatilize drive oil, which builds up in the chamber during runs near room temperature. The use of settings much in excess of 80 V for a system similar to the present one is not recommended, however, as coil temperatures in excess of the thermoplastic threshold for Teflon may be reached.
DISCUSSION

For some applications in measurements of sedimentation velocity, convection may be avoided by use of the band sedimentation technique introduced by Vinograd et al. (13), in which a thin lamella of solution is automatically layered onto a solvent such as 3 M CsCl or 50% H$_2$O-D$_2$O. The motion of the sedimenting band is protected from convection by the density gradient produced in the solvent. Rosenbloom and Schumaker (14) carried out boundary sedimentation runs using performed sucrose gradients to protect against convection.

However, sedimentation velocity measurements may be desired where the three-component systems necessarily involved in the above methods are not present, thus avoiding three-component corrections. Such experiments must be performed under conditions in which the boundary motion is unaffected by convection. It is seen that a temperature control system such as that described above enables such measurements to be made. Also, the modified high-temperature system allows such sedimentation runs to be done at elevated temperatures. Reliable data have been obtained as high as 60°C in this laboratory but a number of 60°C attempts have been ruined by convection. Temperatures near 50°C have been used with consistently good results. The advantages of titanium centerpieces discussed earlier should make construction of titanium centerpieces worth while to many investigators.

ACKNOWLEDGMENTS

The authors wish to acknowledge support from the U. S. Public Health Service Grant GM 11189 and Fellowship 1-P1-GM-32, 419-01 (to Horace Gray). The Laboratory of Chemical Biodynamics provided access to the shops at the Lawrence Radiation Laboratory.

REFERENCES

