Surface modification of Beckman Ultra-Clear® centrifuge tubes for density gradient centrifugation of lipoproteins

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Summary A simple procedure for coating the interior surface of Beckman Ultra-Clear® centrifuge tubes with polyvinyl alcohol is described. The coated tubes are wettable and allow salt solutions to gravity-feed down their sides. This modification of the tubes permits the performance of previously developed density gradient and uniform density ultracentrifugation procedures for lipoprotein fractionation.—**Holmquist, L.** Surface modification of Beckman Ultra-Clear® centrifuge tubes for density gradient centrifugation of lipoproteins. *J. Lipid Res.* 1982. **23:** 1249–1250.

Supplementary key words polyvinyl alcohol • coating • overlaying technique • wettable

The separation of human serum very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) may be conveniently performed in a simple density gradient procedure (1, 2) using a high-speed swinging bucket rotor. In addition, subfractionation of each individual lipoprotein fraction may be performed using the same procedure after modification of the density gradient to suit the relevant presentation of the separation problem.

The use of wettable centrifuge tubes is fundamental for the accomplishment of this gradient centrifugation technique. Cellulose nitrate tubes (Beckman) have been used successfully. However, we have been informed that the factory has ceased to make these tubes, which will be replaced by tubes composed of a hydrophobic plastic (Beckman Ultra-Clear®, nonwettable; producer's specification). This material does not allow the salt solutions of decreasing densities to gravity-feed down the side of the centrifuge tube, which is essential for avoiding mixing at density junctions and at salt-sample interfaces in the work with lipoproteins.

The present communication reports a simple and rapid procedure for coating the interior surface of Beckman Ultra-Clear® centrifuge tubes with a thin film of polyvinyl alcohol, which permits the performance of previously described density gradient procedures (1, 2).

Materials and methods

Beckman cellulose nitrate centrifuge tubes and Ultra-Clear® centrifuge tubes (14 × 95 mm) were purchased from Beckman Instruments Inc., Palo Alto, CA.

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; EDTA, ethylene diamine tetraacetic acid.

Polyvinyl alcohol (mol wt 72000) was obtained from Merck-Schuchard, Germany. All organic solvents used were of analytical grade.

Blood from healthy donors was collected by venipuncture in the morning after an overnight fast and was allowed to clot at room temperature for 2 hr. Solutions containing 270 mmol/l EDTA and 40 mmol/l Merthiolate were then added to a final concentration in the serum of 1.4 and 0.25 mmol/l, respectively.

Procedure

Polyvinyl alcohol (2 g) was dissolved in distilled water (50 ml) by stirring and heating to gentle reflux. Isopropanol (50 ml) was slowly added to the hot solution and stirring and heating was continued until a clear solution was obtained. Finally the solution was allowed to cool to room temperature.

The Beckman Ultra-Clear® tubes were then filled with this coating solution which was aspirated out by means of a water pump after 15 min, leaving a thin film on the tube walls. A small amount of solution collected in the bottom of the tubes on standing which was eventually removed about three times with a Pasteur pipette. The treated tubes were left open to dry at room temperature overnight. The tubes were then filled with distilled water which was poured out after standing overnight at room temperature. Finally the tubes were briefly flushed with water, tapped to remove excess of liquid, and left to dry.

Results and discussion

In order to form density gradients in the untreated Ultra-Clear® tubes, it is necessary to use an underlaying technique (producer's recommendation) in which the more dense salt solution is injected with a hypodermic needle beneath the less dense one. The plasma or serum sample finally has to be placed, by injection, underneath the salt gradient in the bottom of the centrifuge tube. In this procedure, working with lipoproteins, it is difficult not to contaminate the formed gradient with the sample when pulling up the needle through the gradient. In addition, this underlaying technique is laborious and time consuming.

After coating the interior surface of the Ultra-Clear® tube with a thin layer of polyvinyl alcohol, however, a discontinuous density gradient can be easily formed by overlaying salt solutions of decreasing densities above the plasma or serum sample in the bottom of the tube, as described by Lindgren, Jensen, and Hatch (1) and Redgrave, Roberts, and West (2). The salt solutions were allowed to gravity-feed down the side of the wettable centrifuge tube.

Less than 100 μg of coating material is consumed per tube weighing 3 g, making the increase in weight neg-

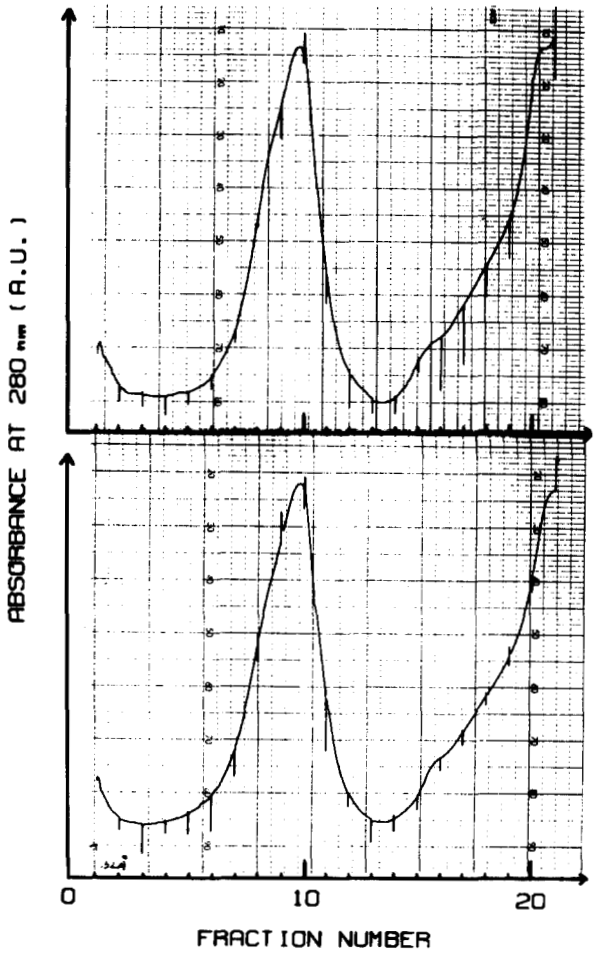

Fig. 1. Absorbance at 280 nm of the effluent from the top of a cellulose nitrate tube (upper panel) and a polyvinyl alcohol-Ultra-Clear® tube (lower panel) after ultracentrifugation of a serum sample in a density gradient 1.100–1.006 kg/l according to Carlson and Redgrave (10). The contents of the tubes were pumped out in 0.5-ml fractions by introducing Maxidense™ through a needle in the bottoms of the tubes. The first 0.5-ml fraction in the top of the tubes containing VLDL was removed with a pipette before pumping started. The peak in the middle of the curves is purified LDL followed by serum proteins. A.U., arbitrary units.

ligible. No effect of the coating procedure on the mechanical or optical properties of the Ultra-Clear® tubes could be found.

The washing procedure of the coated tubes, as described in Methods, eliminates excess of polyvinyl alcohol that otherwise might be dissolved in the salt solutions of the gradient. The residual thin coat of polyvinyl alcohol sticks very firmly to the tube walls and the tubes remain wettable even after being left overnight filled with salt density solutions of 1.25 kg/l.

Polyvinyl alcohol at a concentration of 10 g/l in a VLDL preparation had no effect on blanks or samples in the determination of triglycerides (3, 4), cholesterol (5), cholesteryl esters (6), and phospholipids (7). In addition, this concentration of polyvinyl alcohol in VLDL, LDL, and HDL preparations did not affect the agarose gel electrophoresis of serum lipoproteins according to Noble (8). However, the polyol produced color in the Lowry et al. (9) assay of proteins, but at a concentration of 15 mg/l of the polymer in isotonic sodium chloride, a blank was obtained that did not differ from the normal blank in the assay utilizing a protein standard ranging 75–900 mg/l. Analysis of a 1.100–1.006 kg/l salt density gradient formed after ultracentrifugation in a polyvinyl alcohol-coated Ultra-Clear® tube, with all analytical methods mentioned above, demonstrated the complete absence of interfering material.

Fig. 1 shows the result of a density gradient separation of LDL from human serum as described by Redgrave and Carlson (10) in Beckman cellulose nitrate ultracentrifugation tubes and in polyvinyl alcohol-coated Ultra-Clear® tubes.

We believe this modification of the Beckman Ultra-Clear® centrifugation tubes to be relevant to the progressing work on lipoproteins.

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