

Rapid, High-Volume Fractionation of Plasma Proteins in a Thermo Scientific Reorienting Gradient Zonal Rotor

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Introduction

Investigators involved in lipoprotein research have, for the most part, used the fixed angle rotor and repeated centrifugations to isolate the main plasma lipoprotein fractions.^{1,2} However, the disadvantages of this sequential floatation technique are:³

- long centrifugation times
- tube and tube cap expense
- wear and tear on the ultracentrifuge

We have developed a technique for the single spin isolation of large amounts of major plasma fractions using the Thermo Scientific TZ-28 Zonal Rotor. The technique was developed with the cooperation and advice of the Sorvall Applications Laboratory. The process was developed in an effort to increase the volume output of LDL (low density lipoprotein) while maintaining a good protein concentration and the same or better quality than that obtained using a fixed angle rotor and sequential floatation.

Methods

Plasma Collection

Venous blood was collected from healthy human subjects who had fasted for 12 hours prior to donation. This fasting period eliminated additional centrifugation needed to remove chylomicrons,³ otherwise present in significant amounts in non-fasting individuals. Blood was collected in 600 mL blood bags (Fenwal Laboratories, Deerfield, IL 60015). 7.5 mL of a sterile solution of 0.25M disodium EDTA, pH 7.4 was added to each bag. Plasma was separated by low speed centrifugation of the whole blood collected and stored at 4°C. Further fractionation and separations were carried out within 24 hours.

Zonal Procedure

The total volume capacity of the TZ-28 Zonal Rotor is 1350 mL. A simple discontinuous (0.9% NaCl, pH 7.4) density gradient was used. The plasma from 2 donors was pooled

and a volume of 450 ml was used. The initial density of the plasma, 1.006 g/mL, was adjusted to a final density of 1.3 g/mL by adding solid potassium bromide (KBr). The density adjustment was made using the Radding-Steinberg formula given below:⁵

$$X = \frac{V(d_f - d_i)}{1 - (0.312 \times d_i)}$$

where X = g of KBr; d_f = final density, d_i = initial density, V = volume of plasma in mL, and 0.312 = partial specific volume of KBr.

The TZ-28 was loaded statically using a peristaltic pump. 800 mL of the 0.9% NaCl was pumped into the rotor at a rate of 20-25 mL per minute. The pump was stopped, leaving the tubing full of the saline solution (done so as not to introduce bubbles into the gradient). Following this 500 mL of plasma (actual volume

Figure 1 — Cholesterol Distribution

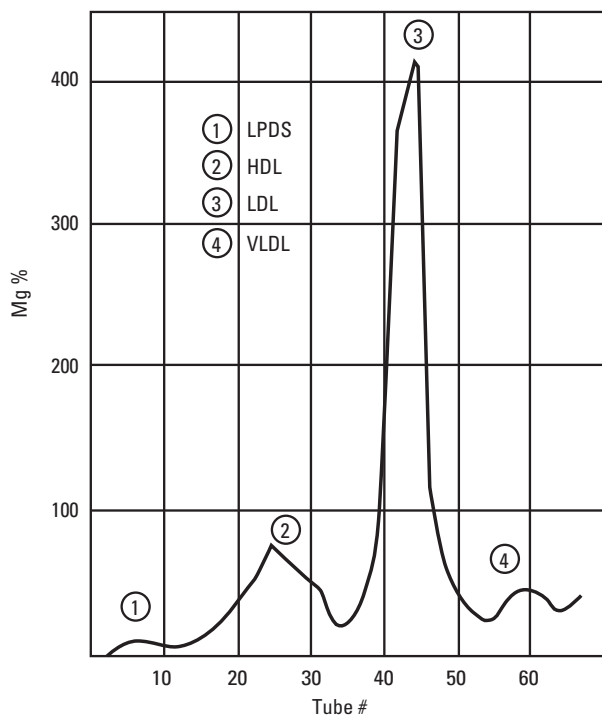


Figure 2 — Triglyceride Distribution

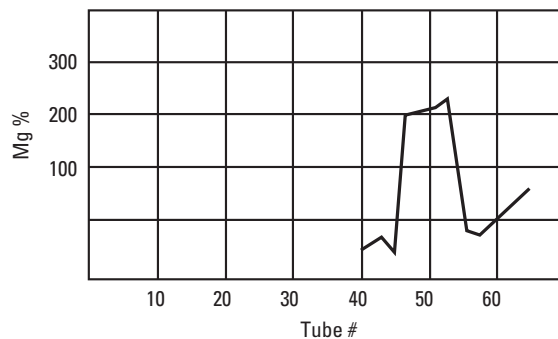


Figure 3 — Density

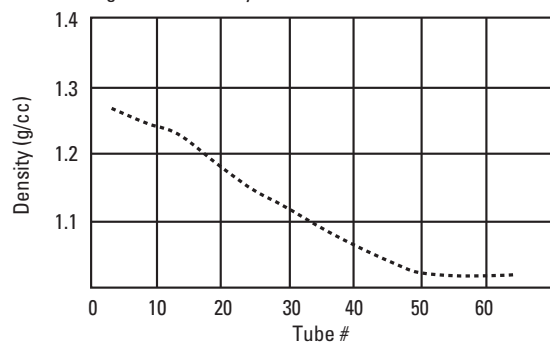


Figure 4 – Absorbance (280 nm)

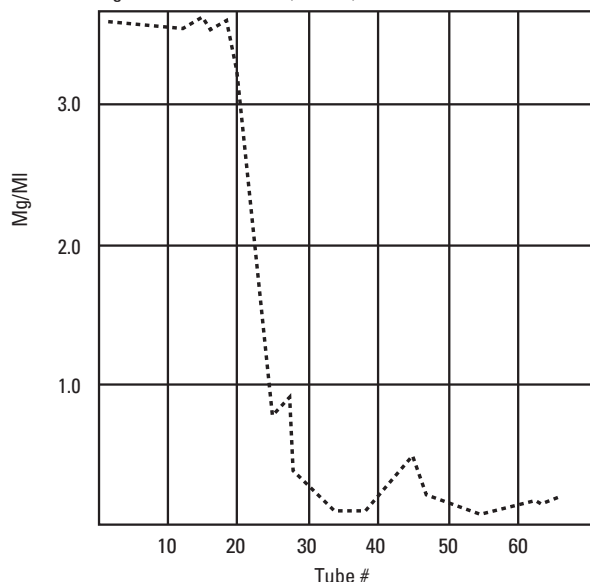
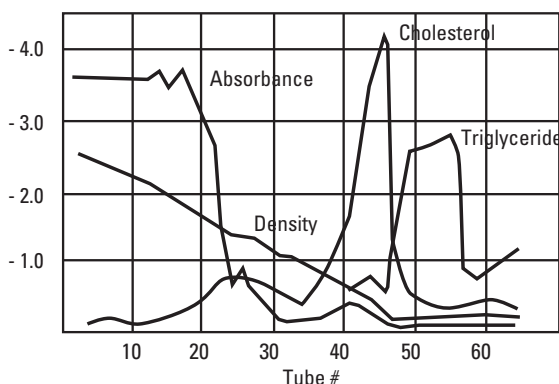


Figure 5 – Summary



plasma are shown in Figures 1 and 2, respectively. Density and absorbance curves are

plotted in Figures 3 and 4, respectively. Figure 5 combines all curves showing their correlation.

After repeated zonal runs, fractionation was so consistent that the LPDS and HDL fractions were collected in a graduated cylinder (410 mL and 210 mL, respectively) rather than in a fraction collector. The LDL fraction was collected using a fraction collector and the 3 peak tubes were pooled.

Because all major fractions are separated after one spin using this procedure, each fraction can be washed and/or dialyzed according to the method of Havel, et. al.¹ The volumes of the 4 main plasma fractions are approximately doubled. The washed LDL fraction protein concentration ranges from 20-30 mg/mL as determined by the Lowry protein assay, absorbance at 670 nm.

A comparison of final LDL product

fractionated by both the single-spin, zonal rotor technique was carried out. Three bags of plasma were pooled and 450 mL fractionated using the zonal rotor. The remainder of the pooled plasma was fractionated using fixed angle rotor.¹ The LDL fraction obtained by both techniques was analyzed using a 15% electrophoresis slab gel by the method of Laemmli.⁶ As shown in Figure 6, the electrophoresis pattern of the LDL prepared using the single-spin, zonal rotor technique compares favorably with that of the LDL prepared using the fixed-angle rotor.

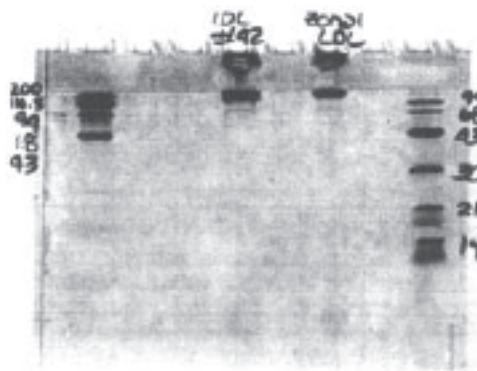


Figure 6 - Electrophoresis Patter, LDL Fraction
Lane 1 and 8 - molecular markers, Lane 4 LDL fraction prepared with fixed-angle rotor, Lane 6 LDL fraction prepared using single-spin, zonal rotor

Conclusion

The TZ-28 reorienting gradient zonal rotor provides a fast and efficient method for large scale fractionation of plasma proteins while maintaining the highest quality and good protein concentration. This is

with KBr added) was pumped into the rotor at an initial flow rate of 10 mL per minute for the first 5 minutes and then the flow rate was increased to 20-25 mL per minute. The plasma (adjusted density of 1.3 g/mL) flowed into the TZ-28 and layered underneath the saline solution. The TZ-28 was run in a Thermo Scientific Sorvall® WX ultracentrifuge at 28,000 rpm (83,500 x g) for 21 hours at 4°C. A rate control setting of 4 was used to carefully control the acceleration/deceleration phase to insure proper reorientation of the gradient.

RESULTS

The rotor was unloaded statically into 20 mL tubes mounted on a timed fraction collector. The fractions were easily distinguishable by colour: (LPDS) lipoprotein deficient serum, usually a light to medium green; (HDL) high density lipoprotein, yellow; (LDL) low density lipoprotein, bright orange; and (VLDL) very low density lipoprotein, milky white.

The distribution of cholesterol (Boehringer-Mannheim cholesterol colorimeter test, absorb at 410 nm) and triglyceride (Boehringer-Mannheim enzymatic assay, absorb at 340 nm) within the fractionated

particularly true for the LDL fraction. Table 1 compares the processing time for fractionation of equal volumes of plasma using the technique described here and the classical fixed-angle procedure.

The obvious conclusion is that where constant, large volumes of plasma fractions are needed or desired, the TZ-28 zonal rotor is both a time and money saving alternative to the fixed-angle technique.

Table 1
Processing Times Using Fixed-Angle and Zonal Rotor Techniques
(All Figures Are Calculated Using 2 Bags of Plasma)

	Fixed-Angle Method	Zonal Method	
1st Floatation Spin (x2)	40 hrs.	Zonal Rotor Spin	21 hrs.
2nd Floatation Spin (x2)	48 hrs.	LDL Wash	18 hrs.
LDL Wash Spin (x2)	36 hrs.	LPDS (Spin Complete)	
LPDS Separation Spin	36 hrs.	HDL	24 hrs.
HDL Separation Spin	24 hrs.		
Total 184 hrs. or 7.6 days		Total 63 hrs. or 2.6 days	

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References

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