

MEASUREMENT OF LIPOPROTEINS USING THE ZETASIZER 3000 BY P. HARRIP¹ AND DR. D. O'NEAL²

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INTRODUCTION

The particle size of Low Density Lipoproteins (LDL) is a significant clinical indicator of cardiovascular disease. For instance, LDL particles display heterogeneity in size and density, which has previously been well documented. This heterogeneity has clinical relevance, in that small, dense LDL particles have been demonstrated to be associated with an increased risk of coronary heart disease.



The traditional method for measuring the particle size of LDL is by Gradient Gel Electrophoresis (GGE). However, this method is slow with results after sample preparation typically available after 20 hours. The Gels are also becoming quite expensive.

A major problem with the GGE method is the dependence on Gel characteristics, which unfortunately can be quite variable.

A method using Photon Correlation Spectroscopy (PCS) was proposed for the measurement. A major advantage of PCS is that the measurement is based on first scientific principles, so has an inherently high level of repeatability within the constraints of sample preparation and presentation.

Photon Correlation Spectroscopy:

The use of Photon Correlation Spectroscopy (PCS) for measuring LDL was first proposed in the 1970's, but it is only recently that instruments have become stable enough to enable their routine application to this measurement.

Essentially, Photon Correlation Spectroscopy can be described as the measurement of the velocity of particles diffusing due to Brownian motion. The instrument measures the Diffusion Coefficient (D) and converts this to size (S) using the Stokes-Einstein equation:-

$$D = \frac{kT}{3\pi\eta S}$$

Malvern Zetasizer 3000:

We have recently used the Malvern Zetasizer 3000 to study LDL particle size and compare the results with the traditional GGE method.

The Zetasizer 3000 was chosen because of the precise temperature control available with this instrument. The range of LDL size over which measurements are made is from about 22nm to 25nm. To accurately measure and resolve the size differences between particles in this size range requires a stable system, particularly very accurate and precise temperature control.

The Zetasizer 3000 has such a controller, and it is this breakthrough in technology that has provided the degree of accuracy necessary for the clinical measurement of LDL.

The actual measurement method is described in a paper, Ref 1.

Repeatability of the measurement is shown in Figure 1. This is a display of 10 repeat runs on the same sample, and indicates a repeatability of 22.8 ± 0.1 nm.

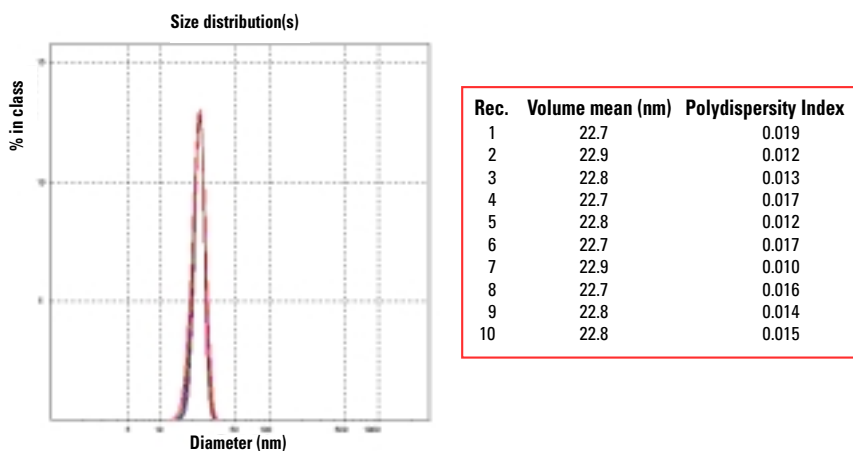


Fig. 1: Overplot of 10 measurement results and table

While the measurement of LDL using Gradient Gel Electrophoresis provides results that are slightly larger than PCS, this is probably due to the way interpretations of particle size are made from different physically measurable phenomena. After sample preparation, results by PCS are typically available after 30 minutes.

Results:

Results of the study are shown in Figure 2. The scattergram indicates the size as measured by GGE compared to the size measured by the Zetasizer 3000. With a 'p' value of <0.0001 and an 'r' value of 0.78, the two sets of data can be said to correlate well.

Conclusion:

The temperature controller in the Zetasizer 3000 has provided the control capability to accurately measure Low Density Lipoprotein particles quickly and with a lower propensity for error than GGE. Use of this instrument would be of great advantage of quick turn-around time, which can be of great advantage both in the clinical diagnostic laboratory and in research. However, the major advantage of the technique is its independence of Gel Quality. The technique and the instrument also have potential for sizing of other Lipoprotein classes.

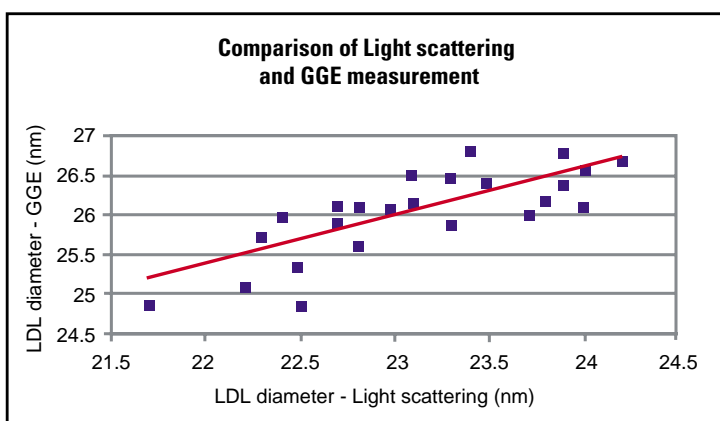


Fig. 2: Comparison of light scattering and GGE measurement

Ref 1. A comparison of LDL size determination using gradient gel electrophoresis and light scattering methods.

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