

Abstract

The disorders of the lipid metabolism have to be monitored for a number of socially significant diseases. For the diagnosis of these disorders, it turned out in the last 10 years as very beneficial to determine the particle size of the individual lipoprotein classes, in particular the low-density lipoprotein. Among several known methods, we have chosen gel filtration with the light scattering detector.

Lipoprotein fractions were separated by ultracentrifugation from the plasma of four donors. Three biochemical parameters – total cholesterol, triacylglycerol, and total protein – were routinely determined for plasma and for each fraction. The silica column BioBasic SEC 300x7,8 mm was calibrated by the proteins thyroglobulin, ovalbumin and angiotensin II with UV detection (280 nm). These proteins are not suitable (due to the size) for the calibration method SEC-LLSD. Therefore, latex beads of various modifications and sizes were tested. The only usable measurement was obtained for the deep-blue latex beads of the size 55 nm, the others remained retained in the column. The lipoprotein fractions of VLDL and LDL were examined by the SEC-LLSD method at different pH (7.3, 7.5, 7.7, 8.0) and ionic strength (0.1M phosphate buffer, 0.1M phosphate buffer and 0.9% NaCl, 0.05M phosphate buffer and 0.9% NaCl). The optimal mobile phase is the 0.1M phosphate buffer and 0.9% NaCl. Further, the mixture of the VLDL and LDL fractions was analyzed, which yielded two imperfectly divided peaks. To determine the average molar mass, this distribution would be acceptable. However, to analyze the quantitative relations more accurately, a more precise distribution would be needed. It could be achieved by combining multiple columns in a series or by selecting a different stationary phase.