

Analytical Ultracentrifugation Analysis

By J. Frederick

Centrifugation is a useful method for the separation and analysis of biomolecules. Analytical ultracentrifugation analysis (also referred to as sedimentation equilibrium) is a technique used to determine the aggregation state of a protein or peptide sample – whether it exists as a monomer, a dimer, a trimer, and so on.

The principle behind this method is based upon the mathematical description of how a particle behaves when subjected to a centrifugal force. The sedimentation velocity (how fast a particle moves toward the bottom of a tube) depends on factors including the mass, shape and density of the particle.

Gradient centrifugation is used for the separation of proteins with different sedimentation coefficients. A linear density gradient is formed by mixing high and low density solutions in a centrifuge tube. A solution containing the proteins to be separated is then layered on the top. As the rotor is spun, the proteins move through the solution to separate at rates dependent upon their sedimentation coefficients. The separated bands of protein can be collected for analysis by piercing a hole through the bottom or side of the tube and carefully withdrawing drops of solution.

Sedimentation equilibrium is used for the direct determination of the mass of a protein. Samples are centrifuged at low speeds to counterbalance sedimentation with diffusion. This method of mass determination is highly accurate and can be used under non-denaturing conditions to preserve the native quaternary structure of multimeric proteins. By comparing the estimated mass of denatured polypeptide chains elicited from SDS-polyacrylamide gel electrophoresis to the mass of the intact protein determined by sedimentation equilibrium analysis, one can ascertain how many copies of each polypeptide chain are in the protein. This method can be used to determine whether a peptide exists as a monomer, or whether it dimerizes or forms higher aggregates in solution.

The Schepartz laboratory has established a collaboration with Jim Lear at the University of Pennsylvania for analytical centrifugation analysis.