AUC FUNDAMENTALS

Forces on Particles

\[
\text{Lamm Equation: } \frac{dr}{dt} = \frac{S^2 r}{D} \left( 1 - \frac{C}{2D} r^2 - \frac{1}{3} r^3 \right)
\]

Components of an AUC Cell

- Two-sector centerpiece sandwiched between two quartz or sapphire windows & assembled into a cylindrical housing.
- A screw ring is torqued to seal the cell.
- A counterbalance is necessary.

AUC WORKFLOW CASE STUDY WITH INSULIN

Comparison of ProteomeLab vs Optima AUC

Experimental Setup

40,000 rpm with 200+ absorbance scans (280 nm)

Data Edits

Eliminate spikes, define the meniscus, and limit radial range

Data Processing

Process data via finite element methods and filter out noise

Plot & Interpret

Interpret results from population distributions vs. MW or model-free graphical interpretation plots

QUALITY CONTROL & CHARACTERIZATION USING AUC

Quantification of Aggregation and Degradation of Biological Samples and Elucidation of Molecular Shape

BSA Concentration Distribution

Liposomes 2D Distribution

INTRODUCTION

Ultracentrifuges spin samples with centrifugal forces typically spanning 100,000 – 1,000,000 x g. At these high forces, the constituent molecules in the sample separate based on their physical properties (e.g., size, mass, density, anisotropy). Accordingly, ultracentrifugation is commonly used to purify, as well as characterize, low-molecular weight polymers up to multi-megaDalton protein complexes and organelles.

PREPARATIVE ULTRACENTRIFUGATION

Differential Ultracentrifugation

- Particles are separated on the basis of their size and mass (sedimentation coefficient, S).
- Multiple pelleting steps may be used for iterative enrichment.
- Ideal for separating particle groups of very different sizes.

Density Gradient Ultracentrifugation (DGUC)

- Soluble particles are separated in a liquid column of varying density (density gradient).
- In rate zonal experiments, particles migrate at varying rates, dictated by their S-values, and are time-dependent.
- Isopycnic separations are time-insensitive, where particles migrate to their apparent buoyant density in the gradient.
- Ideal for high-resolution separation of small materials with similar physical properties.

Example Workflow for DGUC Purification of Plasmid DNA

- Plasmid DNA may be extracted from bacteria using a variety of methods.
- The workflow below depicts a common alkaline lysis extraction and purification via a cesium chloride (CsCl) density gradient method with ethidium bromide.
- Newer density gradient materials (i.e., iodixanol/OptiPrep) and DNA-interacting probes (e.g., DAPI & GelGreen) may also be used in plasmid purification.

AUC HISTORY

Theodor Svedberg invents AUC; Chemistry Nobel Prize 1928

Jean Perrin describes Sedimentation Equilibrium, Physics Nobel Prize 1926

Ole Lamm describes the sedimentation and diffusion of samples in a sector-cell, 1930s

Edward Pickard starts Spinco and builds the first commercial AUC – the Model E, 1947

Beckman acquires Spinco, 1954

Mestton & Stahl experiment, 1958

Schachman develops Reyligh Interference detection, 1958

Van-Hoacle Velchot graphical analysis is developed, 1978

Beckman introduces the ProteomeLab XLA/AFC AUC instrument, 1990s

Demeter develops Ultracent, 1998, Shuck develops SediFit, 2000

Beckman releases the new generation Optima AUC with multi-wavelength capability

New frontier: Viral vectors, nanoparticles, liposomes

REFERENCES:


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