

Bradford Assay



General Aim

To measure the concentration of proteins in a solution by colorimetric analysis using Bradford assay.

Method

Protein concentration measurement according to Bradford assay method

Learning Objectives (ILOs)

- Identify the amino acids that the Bradford Protein Assay measures.
- Describe the color change that occurs when proteins combine with Coomassie dye under acidic conditions.
- Illustrate the correct standard curve equation for an example BSA standard.
- Interpret the standard curve equation when given example data.
- Calculate the protein concentration of an example.
- Recall the substance commonly used as standards in the assay and the device used to measure the color of the samples.

Theoretical Background / Context

-Bradford's assay is Colorimetric. It's based on color changes that occur when the protein in the sample interacts with a specific reagent causing color changes that can be measured using a spectrophotometer.

Theoretical Background / Context (continued)

- Bradford reagent contains the coomassie blue dye. The cationic (unbound) form is green / red with an absorption maximum at 465 nm. The anionic bound form of the dye, held together by hydrophobic interactions, has an absorption maximum at 595 nm. Thus; the increase of absorbance from 465 to 595 nm is directly proportional to the amount (concentration) of protein bound to the dye.

Principle of Work

- The Bradford assay is based on the binding of the basic amino acids of a protein to a dye present in Bradford reagent (e.g. Coomassie brilliant blue). This leads to a shift in the absorbance maximum of the dye from 465 to 595.
- After creating a standard curve of protein solutions with known concentrations, the protein concentration of unknown samples can be plotted on the standard dilution curve and calculated.