Protein quantification assay using the Bradford method

Laxman Manjhi 2019BB10034

Ratnesh Kumar Sharma 2019BB10047

Background

- The Bradford protein assay, also known as the Bradford method, is a widely used technique for quantifying the amount of protein in a biological sample.
- The assay is based on the principle that the absorbance of a solution changes when a protein is present.
- The assay was developed by Marion M. Bradford in 1976.
- The Bradford assay uses the dye Coomassie Brilliant Blue G-250, which binds to proteins in a sample and causes a change in the dye's absorbance.
- The absorbance is measured at a wavelength of 595 nm using a spectrophotometer.
- A standard curve is generated using known concentrations of a protein, and the absorbance of the unknown sample is used to determine its protein concentration.
- The Bradford assay is a quick, simple, and sensitive method for protein quantification.
- It is also relatively inexpensive and can be performed in a small volume of sample.
- However, it is not specific for certain type of proteins, and not all proteins bind to the dye equally, so it may not be suitable for certain types of samples or proteins.

Method

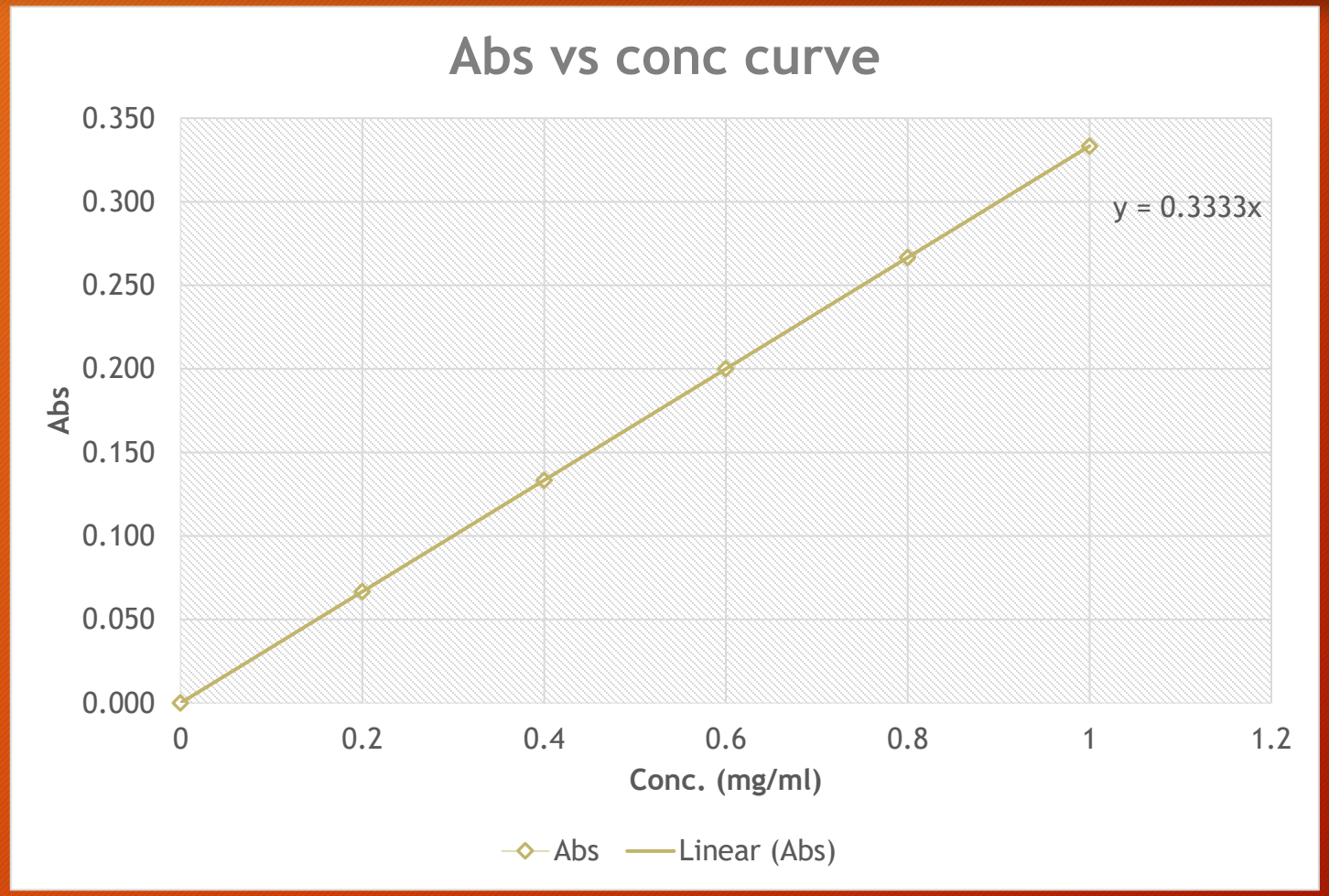
The Bradford protein assay was performed as follows:

- There was protein standard solution of known concentration.
- A series of dilutions of the protein standard solution, ranging from a high concentration to a low concentration, were prepared.
- A small amount of the Bradford reagent, Coomassie Brilliant Blue G-250, was added to each of the standard solutions and the unknown sample.
- The solution was mixed well and incubated for 5-10 minutes to allow the dye to bind to the proteins.
- The absorbance of each solution was measured at a wavelength of 595 nm using a spectrophotometer.
- The absorbance of the standard solutions was plotted against their protein concentration to create a standard curve.
- The standard curve was used to determine the protein concentration of the unknown sample by comparing its absorbance to the absorbance of the standard solutions.

Observation

Concentration	Observation			
(mg/ml)				Avg
0	0	0	0	0.000
0.2	0.139	0.149	0.148	0.145
0.4	0.377	0.363	0.367	0.369
0.6	0.433	0.437	0.494	0.455
0.8	0.497	0.5	0.495	0.497
1	0.556	0.548	0.538	0.547
Unknown 23	0.372	0.375	0.376	0.374
Unknown 36	0.595	0.595	0.602	0.597

Standard curve



Concentrations of unknown samples

- Concentration of unknown 23

  = 0.374/0.3333

  = 1.123 mg/ml

- Concentration of unknown 36

  = 0.597/0.3333

  = 1.792 mg/ml

Thank you