

Spectrophotometric methods for determination of proteins concentration

Determination of proteins using three spectrophotometric methods:

1. Bicinchoninic acid (BCA, Smith) Method.
2. Bradford Method.
3. Warburg-Christian Method (A280/ A260 Method).

Qualitative test

refers to descriptions or distinctions based on some quality or characteristic rather than on some quantity or measured value. It can be a form of analysis that yields the identity of a compound.

Quantitative

refers to a type of information based in quantities or else quantifiable data.

-In this lab three of these methods will be studied. Two are newer methods which are widely used and one is an older method.

-In the newer methods chemical reagents are added to the protein solutions to develop a color whose intensity is measured in a spectrophotometer.

-The third method relies on direct spectrophotometric measurement.

1. Bicinchoninic acid (BCA, Smith) Method:

-The method has a high sensitivity, as low as 1 μg protein can be detected.

Principle:

The **purple color** resulting from this method is due to:

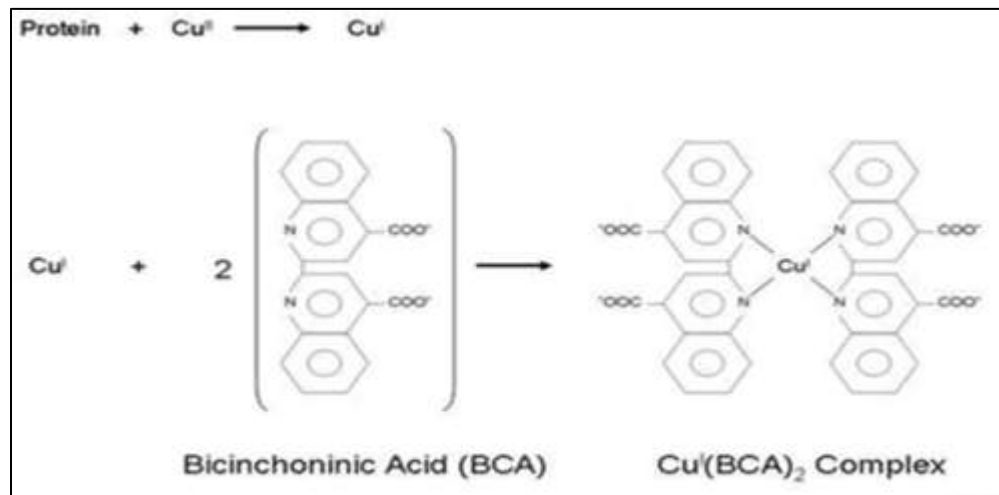
A] The nitrogens in peptide bonds in protein, reduce Cu^{2+} ions
 \rightarrow to Cu^+

(a temperature dependent reaction) under alkaline conditions.

[reduction reaction]. the formation of a Cu^{2+} -protein complex in a basic environment, followed by reduction of the Cu^{2+} to Cu^+

B] Cu^+ chelated by two molecules of BCA, to produce a copper-BCA complex [purple color] with maximum absorption (λ_{max}) of 562 nm.

concentration



2. Bradford Method:

-This method is based on **protein binding of a dye**, to arginine residues and aromatic amino acid.

-**Fast, accurate and high sensitivity** [1 μg protein can be detected].

-The method is recommended for general use, especially for determining protein content of cell fractions and assessing protein concentrations for gel electrophoresis, about 1- 20 μg protein for micro assay or 20-200 μg protein for macro assay.

Principle:

-Binding of the dye Coomassie Brilliant Blue G-250 to protein in acidic solution causes **a shift in wavelength** of maximum absorption (λ_{max}) of the dye **from 465nm to 595nm**.

-Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible color change.

-The absorbance at 595nm is directly proportional to the concentration of the protein. [The color is stable for one hour.]



Test tubes containing:
Bradford reagent alone.



Test tubes containing:
Bradford reagent with protein added.
(λ_{max}) is shifted to 595nm.

3. Warburg-Christian Method (A280/ A260 Method):

- Is easy, sensitive and fast. It has a sensitivity of about 0.05 - 2.0 mg protein/ml.
- it is not accurate.

Principle:

-This method is based on the **relative absorbance** of proteins and nucleic acids at 280nm and 260nm.

-Tyrosine and tryptophan residues in a protein absorb in the ultraviolet at 280nm. Since the amounts of these residues vary greatly from protein to protein, the method is best used only for semiquantitative analysis of protein samples.

-Nucleic acids which contaminate samples interfere with this method. This problem is overcome by the fact that nucleic acids absorb more strongly at 260nm than at 280nm. while the reverse is true for proteins.

-Calculate the protein concentration in the unknown from the following equations:

1-[A280 x correction factor =..... mg/ml protein].

A280 =.....

A260 =.....

A280/ A260 =.....

Correction factor =.....

Unknown concentration =..... mg/ml

2-or [groves formula]:

Protein concentration [mg/ml]=[1.55 X A280]-[0.76 X A260]

-A protein solution that has a **high** A280/A260 ratio: **Less** contaminated by DNA.

[It shows a lower absorbance at 260nm comparing to absorbance at 280nm].

-A protein solution that has a **low** A280/A260 ratio: **Highly** contaminated by DNA.

[It shows a higher absorbance at 260nm comparing to absorbance at 280nm].