Lab# 2

Spectrophotometric Methods for Determination of Proteins Concentration

BCH 333 [PRACTICAL]

Objectives

Getting familiar with how to determine protein concentration using three spectrophotometric assays:

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

Assays

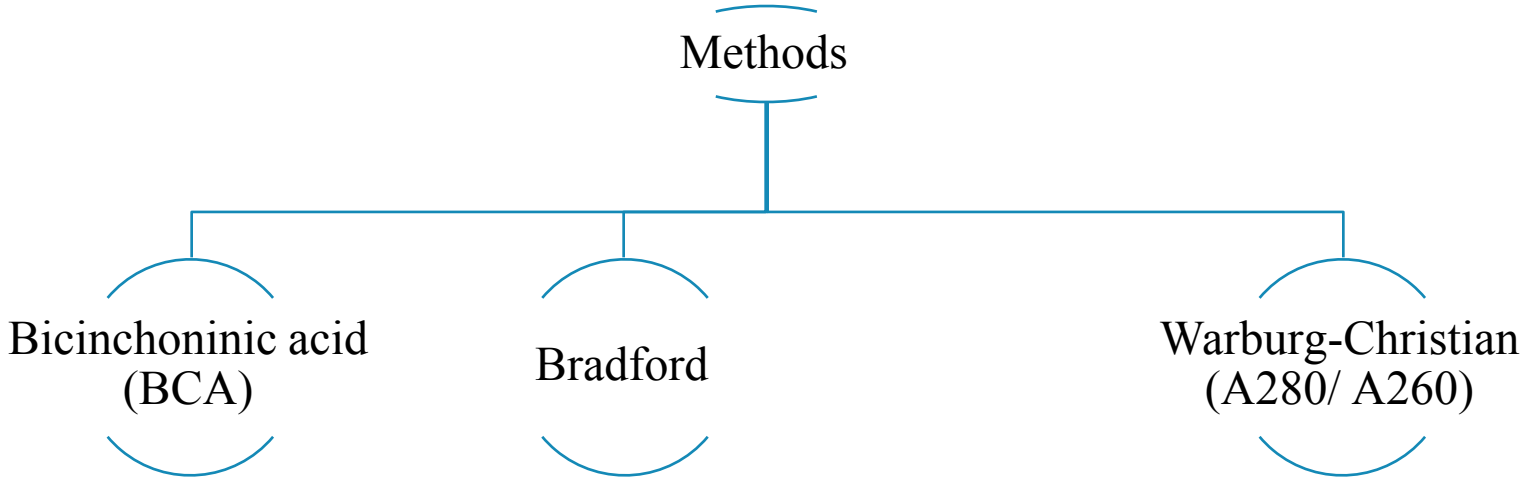
Qualitative

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.

Spectrophotometric Methods for Determination of Proteins Concentration



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

This method relies on direct spectrophotometric measurement.

1. Bicinchoninic acid (BCA, Smith) Method

- High sensitivity [1 µg protein can be detected].
- Slow.

Principle:

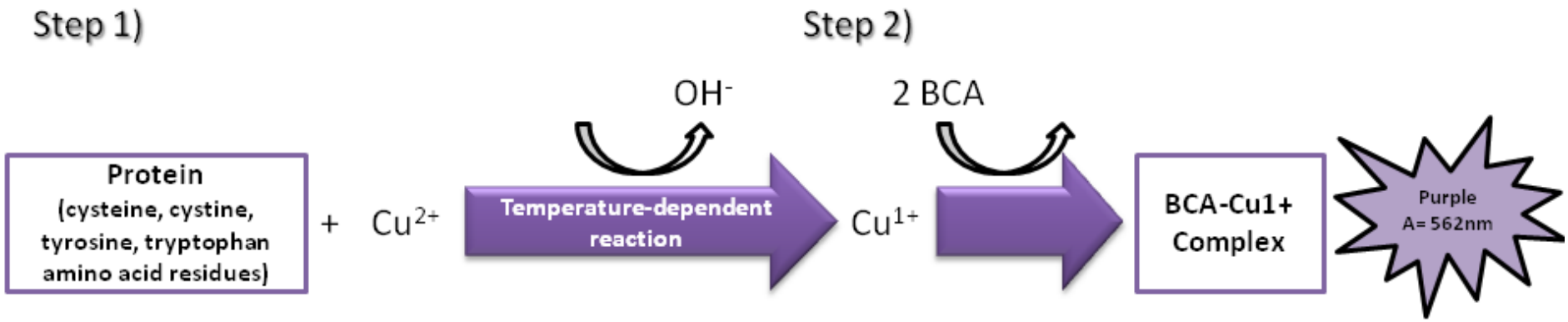
The purple color resulting from this method is due to:

A. **Reduction reaction:** Under alkaline conditions, Cu^{2+} forms complex with the nitrogens of the peptide bonds in protein, this will results in reduce Cu^{2+} ions → to Cu^{+} (a temperature dependent reaction)

B. **Color formation:** the Cu+ will chelated by two molecules of BCA, to produce a (copper-BCA complex) [purple color] with maximum absorption of 562 nm.

1. Bicinchoninic acid (BCA, Smith) Method

Principle continue …



2. Bradford Method

- Fast
- Accurate
- High sensitivity [1 µg protein can be detected].

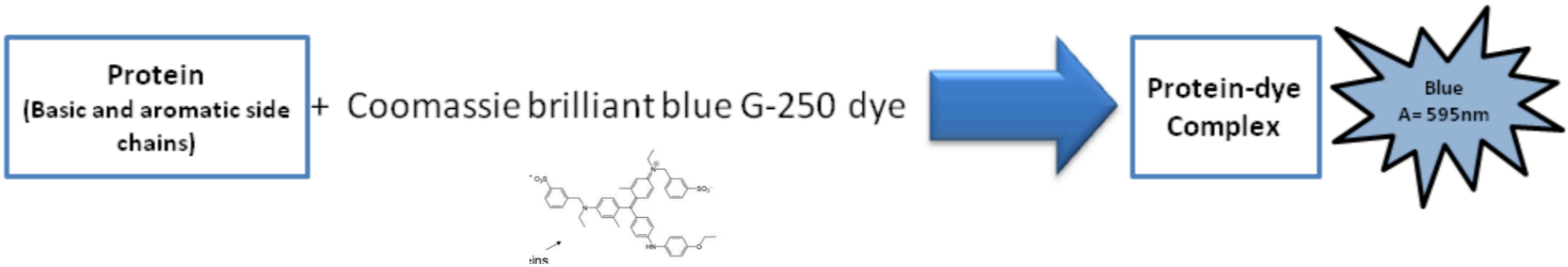
It is for general use:

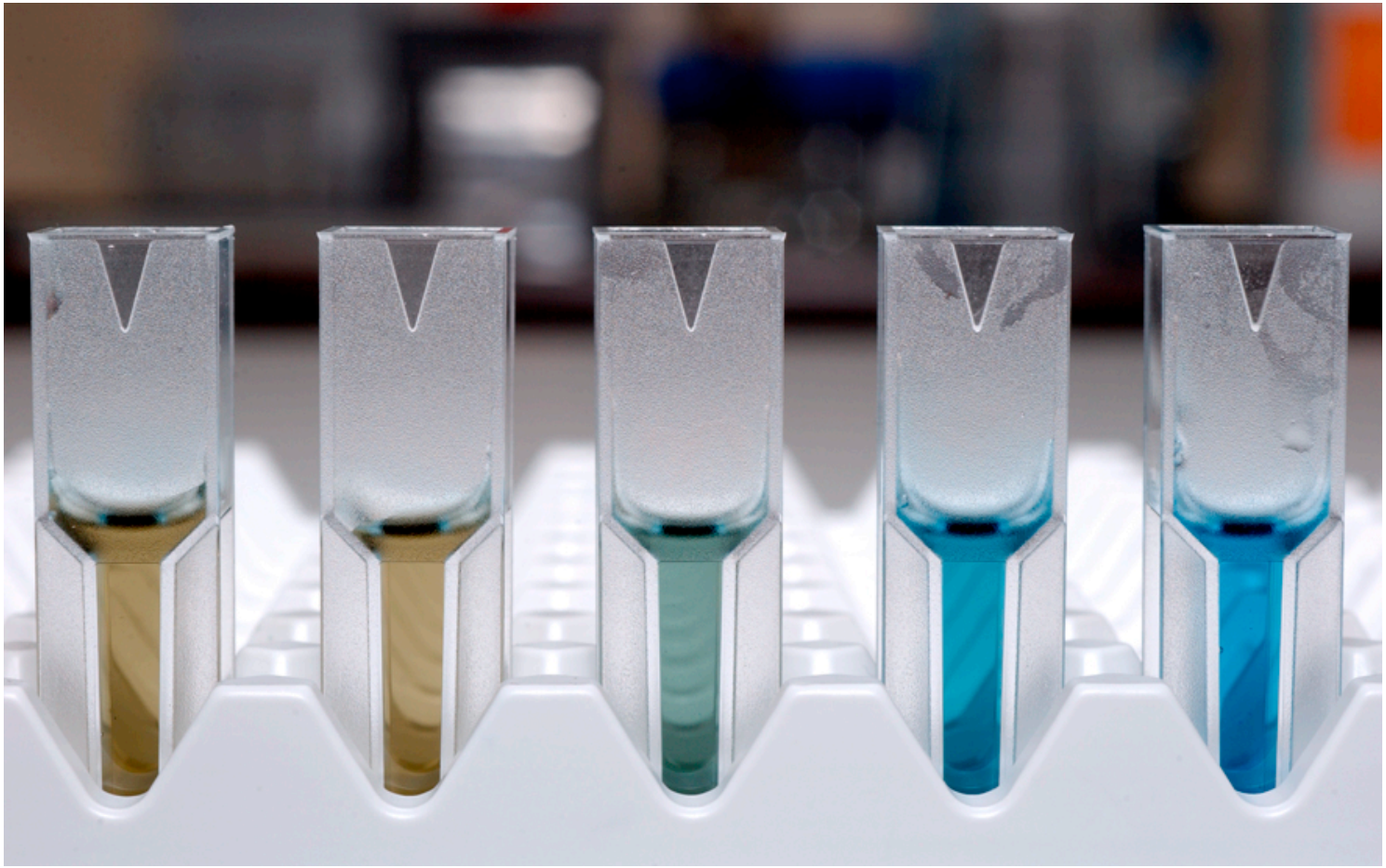
- Determining protein content of cell fractions
- Assessing protein concentrations for gel electrophoresis

2. Bradford Method

Principle:

- Coomassie Brilliant Blue G-250 dye binds to protein (precisely to arginine residues and aromatic amino acid) in acidic solution to form a complex.
- This complex will causes a shift in wavelength of maximum absorption (λmax) of the dye from 465 nm to 595 nm.
- The the anionic form of the dye (complex) is stabilized by hydrophobic and ionic interactions [The color is stable for one hour.]



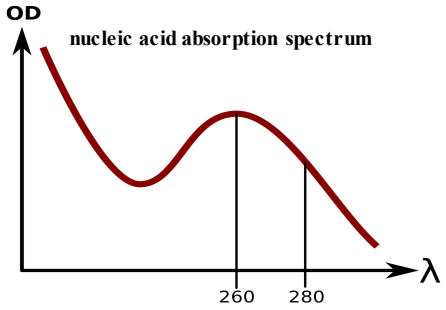


1st two tubes containing:
Bradford reagent alone.
(λmax) of the dye 465nm

The remaining test tubes containing:
Bradford reagent with protein added.
(λmax) is shifted to 595nm.

3. Warburg-Christian Method (A280/ A260)

- Easy and fast.
- It has a sensitivity of about 0.05- 2.0 mg protein/ml.
- Semiquantitative



Principle:

- This method is based on the relative absorbance of proteins and nucleic acids at 280nm and 260nm, respectively.
- Protein can absorb light at 280 nm due to the presence of aromatic amino acid tyrosine and tryptophan. Since the amount of these residues greatly varies from protein to protein this method is semiquantitative.
- Nucleic acids 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.

3. Warburg-Christian Method (A280/ A260)

Calculate the unknown protein concentration by two ways:

1- Protein concentration:
A280 x correction factor = …… mg/ml

A280 =…… **A260** =……
A280/ A260 **ratio** =……
Based on the ratio the **correction factor** =……

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

Or

2- [groves formula]
Protein concentration:
[1.55 X A280]-[0.76 X A260] = …… [mg/ml]

A_{280}/ A_{260} Method

A_{280}/ A_{260}	Correction factor	Nucleic acid (%)
1.75	1.12	0.00
1.63	1.08	0.25
1.52	1.05	0.50
1.40	1.02	0.75
1.36	0.99	1.00
1.30	0.97	1.25
1,25	0.94	1.50
1.16	0.90	2.00
1.09	0.85	2.50
1.03	0.81	3.00
0.98	0.78	3.50
0.94	0.74	4.00
0.87	0.68	5.00
0.85	0.66	5.50
0.82	0.63	6.00
0.80	0.61	6.50

Questions

1) Reaction requires acidic condition?

2) Methods depends on the absorption properties of proteins molecules only in the solution?

3) Bicinchoninic acid method and biuret test are both quantitative tests and depend on reducing Cu+2. [ ]