Bradford Protein Determination of Milk Protein
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Description

It is often necessary to quantitate protein in a biochemistry or biotechnology laboratory. In 1976 Bradford published a rapid and sensitive method for determining the amount of protein in a sample, which has revolutionized protein chemistry. The method had several advantages over previous methods of protein quantitation. The Bradford method is quite fast and convenient and has few of the interferences that many of the older methods were subject to. The Bradford method utilizes the binding of Coomassie® Brilliant Blue G 250 dye to proteins. The dye has both a blue and a red form. When this dye binds to a protein, the red form is converted to the blue form and the absorption maximum of the dye shifts from 465 nm to 595 nm. The binding is very rapid and reproducible. The protein-dye complex remains stable in solution for one hour. Protein samples are mixed with an excess amount of dye and allowed to react for at least two minutes, and then the absorption at 595 nm is measured before one hour. By comparison to protein standards, the amount of protein in an unknown sample can be easily determined. Beer's Law holds for accurate determinations of samples containing 0.5 to 50 μg protein.

The Bradford method can be automated for analyzing large numbers of samples. The Bradford method is subject to interference by detergents such as sodium dodecyl sulfate, Triton X-100, and many common glassware detergents. Care should be taken to ensure that no detergent is present in the glassware used for the protein assay.

Materials
- Coomassie Brilliant Blue G 250
- 95% ethanol
- bovine serum albumin
- whole milk
- chocolate milk
- skim milk
- Spectronic 20
- vortex mixer
- matched cuvettes
- micropipettors and tips
- 0.1 M potassium dihydrogen phosphate (KH_2PO_4)
- 0.1 M sodium hydroxide (NaOH)
- test tubes
- volumetric flasks (10 mL, 100 mL, 1 L)
- deionized water
- vacuum filtration equipment

Safety, Handling, and Disposal

It is your responsibility to specifically follow your institution's standard operating procedures (SOPs) and all local, state, and national guidelines on safe handling and storage of all chemicals and

equipment you may use in this activity. This includes determining and using the appropriate personal protective equipment (e.g., goggles, gloves, apron). If you are at any time unsure about an SOP or other regulation, check with your instructor.

Procedure

Preparation of Coomassie Blue reagent

1. Dissolve 100 mg Coomassie Brilliant Blue G 250 in 50 mL of 95% ethanol.

2. Add 100 mL 85% phosphoric acid (concentrated H_3PO_4).

3. Quantitatively transfer to a 1-L volumetric flask and bring the volume to 1 L with deionized water.

4. Filter the solution by vacuum filtration and store in a reagent bottle until use.

Protein standard determination

NOTE: Prepare the protein standards and the milk unknowns at the same time.

1. To prepare a pH 7.0 phosphate buffer solution, mix 50 mL 0.1 M KH_2PO_4 and 37.1 mL 0.1 M NaOH.

2. Dissolve 10.00 mg bovine serum albumin (BSA) in 10 mL phosphate buffer. Do not shake, since the protein will tend to foam. Keep the solution under refrigeration on ice. It must be stored frozen desiccated below 0°C.

3. Pipet varying amounts of BSA ranging from 10–100 μg to set up three replications each of five different protein standards. Pipet varying amounts of phosphate buffer to make the final volume 100 μL for all standards. Pipet 100 μL phosphate buffer into a tube labeled "blank." Table 1 illustrates typical amounts for a standard curve.

Table 1: Typical Amounts for a Standard Curve		
μL protein (BSA)	μL BSA solution pipetted	μL phosphate buffer pipetted
20	20	80
40	40	60
60	60	40
80	80	20
100	100	0

4. Pipet 6.0 mL Coomassie Brilliant Blue G 250 reagent into each tube and mix using a vortex mixer.

5. Use the tube containing the blank (100 μL phosphate buffer) as the reference to zero the Spec 20 at 595 nm. Read the percent transmittance at 595 nm after 2 minutes and before 1 hour. Convert the percent transmittance to absorbance and average the three absorbance readings for each standard.

Preparation of unknown milk solutions

1. Dilute each milk sample 1:100 in a volumetric flask (i.e., dilute 1 mL of the whole milk to 100 mL with water, dilute 1 mL of the chocolate milk to 100 mL with water, etc.).

2. Pipet 100 μL of each milk solution into a test tube and add 6.0 mL Coomassie Blue reagent to the tube. Prepare three replications of each milk unknown.

3. Determine the percent transmittance for each milk unknown using the blank as the reference. Record the percent transmittance at 595 nm after two minutes and before 1 hour. Convert the percent transmittance to absorbance. Average the three absorbances for each milk unknown.

Preparation of standard curve

1. Plot the absorbance vs. μg of protein for each amount of BSA.

2. Determine the amount of protein in each of the milk unknowns from the graph.

Calculations of total milk protein

1. To determine the total protein in the milk carton, begin with the following relationship:

$$\frac{x\ \mu g\ (from\ graph)}{100\ \mu L\ (volume\ pipetted)} \quad OR \quad \frac{x\ mg}{100\ mL}$$

(remember 1 μg/μL = 1 mg/mL)

$$\frac{x\ mg}{100\ mL} \times 100\ (dilution) \times total\ mL\ in\ milk\ carton \times \frac{1\ g}{1000\ mg} = total\ g\ protein\ in\ milk\ carton$$

2. Compare the total protein calculated to the total protein stated on the carton. Discuss any discrepancies.

3. Repeat for additional milk unknowns.

References

Bradford, M.M. "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Dye Binding." *Analytical Biochemistry*. 1976, 72, 248–254.

Spector, T. (1978). "Refinement of the Coomassie Blue Method of Protein Quantitation." *Analytical Biochemistry*. 1978, 86, 142–146.