Lab sheet #3

-Spectrophotometric Methods for Determination of Proteins Concentration-

When handling Bradford reagent, consider the following:

Always wear gloves, safety goggles, a mask, and a lab coat to prevent skin and eye irritation.

Ventilation: Work in a well-ventilated area, preferably under a chemical fume hood, to avoid inhaling vapors or mists.

Dispose reagent as recommended.

Objectives:

- To determine the concentration of extracted protein by the Bradford method.
- Using the Warburg-Christian method to estimate the concentration of a protein sample.

A. Bradford method:

Method:

1. Set up 9 tubes and label them as follows:

Tube	Bovine Serum Albumin (BSA) (150µg/ml) (ml)	Distilled Water (ml)	Sample with Unknown Concentration (ml)	Bradford reagent (ml)
Blank	-	1	-	5
A	0.07	0.93	-	
B	0.13	0.87	-	
C	0.26	0.74	-	
D	0.4	0.6	-	
E	0.66	0.34	-	
F	1	-	-	
Dialyzed sample	-	-	1	
		0.5	0.5	

2. Mix and incubate at room temperature for 5 min then record the absorbance at 595nm.

Results:

Tube	Concentration (µg/ml)	Absorbance at 595 nm
A		
B		
C		
D		
E		
F		
Dialyzed sample		

- Record the absorbance in the table.
- Calculate the concentration of the standard solutions (A-F) using: C1 x V1 = C2 x V2 formula.
- Plot a standard curve of absorbance at 595nm against BSA protein concentration (µg/ml).
- From the standard curve obtain the protein concentration of dialyzed sample (using TREND formula).

Related questions:

1. Which are the tubes that considered as standard solutions?

   _____

2. What is BSA?

   _____

3. Why did you read the absorbance of the tubes at 595 nm and which type of cuvette did you use?

   _____

   _____

4. Did you dilute your sample? If yes, what is your dilution factor? Write down the calculation.

   _____

   _____

B. Warburg-Christian Method (A_{280}/ A_{260} Method):

Method and results:

1. Read the absorbance of (protein sample A) at 280nm, then read the same sample at 260nm.
2. Record your results:

   - A_{280}= _____
   - A_{260}= _____
   - A_{280} /A_{260}= _____
   - Correction factor from the table= _____
   - **Unknown concentration of protein sample A:**

     A_{280} x correction factor =_____ mg/ml protein.

OR

Grove's formula: Protein concentration= [1.55 X A_{280}]-[0.76 X A_{260}]

= _____ mg/ml

Unknown concentration of protein sample A = _____

Related questions:

1. Can you predict the percentage of the nucleic acid that contaminates the "protein sample A"?

_____

2. Why is this method considered a "direct" assay?

_____

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