First Hope

COMPLETE NOTES

Click here to visit our website

PHARMACY PRACTICAL NOTES

Complete subject and semesters are available on FirstHope app only.

Scan or click here

Click here to get the app >

Available on Google Play

JOIN THE FIRSTHOPE COMMUNITY

Join our Telegram channel

Click here to join Telegram >

Scan or click here

@FirstHopeNotes

Instagram

@firsthope.official

Click here to follow >

YouTube channel

FirstHope-Pharmacy / Live streams

Click here to watch >

WhatsApp channel

Follow FirstHope on WhatsApp

Click here to join >

FirstHope website

firsthope.co.in

Click here to visit >

B.Pharm / D.Pharm / M.Pharm / Pharm.D

Complete subjects and semesters are available in the FirstHope app.

Protein Estimation by Bradford and Lowry Methods

Practical 18 - Analytical practical

Aim

To estimate protein concentration using Bradford or Lowry calibration and suitable matrix controls.

Principle

Bradford measurements depend on dye binding, while Lowry measurements involve copper chemistry and a subsequent colour reaction. Response varies with protein composition and can be affected by detergents, reducing agents and other matrix components.

The test preparation, blank, calibration or titrant standardisation and independent controls are part of the measurement. State the actual concentration units, test conditions and adopted method before interpreting the result.

Materials and Requirements

- Selected Bradford or Lowry reagent method
- Bovine serum albumin or the stated protein reference
- Blank, independent check and suitable readout system
- Qualified balance and volumetric equipment
- Controlled method and raw-data worksheet

Procedure

1. Identify the material, intended attribute, method version and acceptance criteria. Record preparation and instrument or reagent identities.
2. Prepare the prescribed blank and reference or standardised reagent. Verify the measurement with an independent check where applicable.
3. Choose one method for the stated matrix and document its compatible concentration range.
4. Prepare a reference calibration and a separate check in the relevant buffer conditions.
5. Treat standards, blanks and test dilutions using the selected reagent order and timing.
6. Measure at the adopted wavelength, apply the appropriate blank and verify that dilution gives a consistent result.
7. Record individual readings for independently prepared test portions. Keep all raw observations, including unsuccessful measurements and their investigation.
8. Calculate the result on the stated mass, volume or area basis, including dilution, blank and recovery corrections as justified.
9. Compare the evidence with the applicable criteria and write a conclusion limited to the tested preparation and conditions.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Preparation / attribute	Method condition	Raw measurement	Calculated result
Buffer / reagent blank	Record	Record	Calculate

Preparation / attribute	Method condition	Raw measurement	Calculated result
Reference levels	Record	Record	Calculate
Test dilutions	Record	Record	Calculate
Protein-equivalent concentration	Record	Record	Calculate

Calculations

• Protein concentration = concentration obtained from the selected calibration x dilution factor

Worked Example

Illustrative calibration A = 0.400C + 0.020, where C is mg/mL of the reference protein.

A test absorbance of 0.220 gives 0.500 mg/mL in the measured dilution. A ten-fold dilution gives 5.00 mg/mL in the original preparation.

Report the reference and method because responses are not identical for every protein.

Result and Interpretation

• State the method, buffer, reference equivalent and interference assessment.
• Report replicate variability and the evidence that the method can measure the intended attribute in this matrix.

Precautions

• Use compatible buffers and validated assay timing.
• Avoid extrapolation beyond the working range.
• Prepare dilution checks for concentrated or interfering matrices.
• Use the adopted laboratory procedure, suitable controls and the designated waste arrangements.

Viva Questions and Answers

Do Bradford and Lowry have the same chemistry?

No; their response mechanisms and interference profiles differ.

Why identify the reference protein?

Protein composition influences the assay response.

What can a dilution check show?

Unexpected concentration dependence may reveal interference.

First Hope

COMPLETE NOTES

Click here to visit our website

PHARMACY PRACTICAL NOTES

Complete subject and semesters are available on FirstHope app only.

Scan or click here

Click here to get the app >

Available on Google Play

JOIN THE FIRSTHOPE COMMUNITY

Join our Telegram channel

Click here to join Telegram >

Scan or click here

@FirstHopeNotes

Instagram

@firsthope.official

Click here to follow >

YouTube channel

FirstHope-Pharmacy / Live streams

Click here to watch >

WhatsApp channel

Follow FirstHope on WhatsApp

Click here to join >

FirstHope website

firsthope.co.in

Click here to visit >

B.Pharm / D.Pharm / M.Pharm / Pharm.D

Complete subjects and semesters are available in the FirstHope app.