Biochemistry Lab Exam

Protein Quantification by Spectrophotometry

Section A: Multiple Choice Questions (Circle the correct answer)

1. What is a spectrophotometer used for?
A) Measuring temperature
B) Measuring light absorption
C) Measuring pressure
D) Measuring volume

2. The wavelength is measured in:
A) cm
B) nm
C) mol
D) g

3. The UV range is approximately:
A) 400–800 nm
B) 100–400 nm
C) 200–900 nm
D) 50–200 nm

4. Lambda max refers to:
A) Minimum absorption
B) Maximum transmission
C) Maximum absorption
D) Minimum wavelength

5. Beer-Lambert law relates absorbance with:
A) Temperature and pressure
B) Concentration and path length
C) Volume and density
D) Time and speed

6. The unit of concentration (C) is:
A) cm
B) mol/L
C) nm
D) g/cm

7. Absorbance (A) has:
A) Units
B) No units
C) kg units
D) cm units

8. In Beer's law graph, slope equals:
A) C
B) A
C) εl
D) λ

9. At zero concentration, absorbance should be:
A) 1
B) Maximum
C) Zero

D) Negative

10. Calibration curve is used to:
A) Measure temperature
B) Find unknown concentration
C) Measure pH
D) Detect color

11. Bradford assay uses which dye?
A) Crystal violet
B) Coomassie blue
C) Methylene blue
D) Phenolphthalein

12. The optimal wavelength for Bradford assay is:
A) 540 nm
B) 400 nm
C) 595 nm
D) 800 nm

13. BCA assay absorbance is measured at:
A) 595 nm
B) 540 nm
C) 300 nm
D) 800 nm

14. What happens when protein binds to Coomassie dye?
A) No color change
B) Blue color forms
C) Red color forms
D) Yellow color forms

15. The R^2 value should ideally be:
A) Less than 0.5
B) Equal to 0
C) Greater than 0.9
D) Negative

Section B: Problem Solving (Show your work)

1. Given:ε = 200 L/mol·cm, l = 1 cm, A = 0.8.
Find concentration (C).

_____
_____

2. Given: y = 0.0006x + 0.6258 and A = 0.978.
Find x.

_____
_____

Answer Key with Explanation

1) B – Measures light absorption.

2) B – nm is unit of wavelength.

3) B – UV range is 100–400 nm.

4) C – λmax = maximum absorption.

5) B – depends on concentration and path length.

6) B – mol/L.

7) B – no units.

8) C – slope = εl.

9) C – zero concentration $\rightarrow$ zero absorbance.

10) B – used to find unknown concentration.

11) B – Coomassie dye.

12) C – 595 nm.

13) B – 540 nm.

14) B – blue color forms.

15) C – $R^2 > 0.9$ ideal.

Problem Solutions:

1) $C = A / (\varepsilon l) = 0.8 / (200 \times 1) = 0.004$ mol/L

2) $0.978 = 0.0006x + 0.6258$
$0.978 - 0.6258 = 0.0006x$
$0.3522 = 0.0006x$
$x = 587$ µg/mL