Tutorial - Spectrophotometry

1. In an experiment, you need to dilute a stock solution of a compound with a concentration of 0.5 M to achieve a final volume of 1 L with a concentration of 0.01 M. How many ml of the stock solution do you need to use? (20mL)

2. A solution has an absorbance of 0.85 at 400 nm, and the molar extinction coefficient (ε) for the substance at that wavelength is 10,000 L/mol·cm. If the path length of the cuvette is 1 cm, calculate the concentration (in mol/L) of the substance in the solution. (8.5×10^{-5}mol/L)

3. You are studying an enzyme reaction using spectrophotometry. The absorbance at 340 nm decreases from 1.2 to 0.6 over 5 minutes. The molar extinction coefficient for NADH at 340 nm is 6.22×10^3 L/mol. If the path length is 1 cm, calculate the rate of NADH consumption in µmol/min. (96.5 µmol/L)

4. You are performing a Bradford assay to measure protein concentration. A standard curve was constructed, and the equation obtained is y=0.62x+0.05, where y is absorbance and x is the protein concentration in mg/mL. If the absorbance of your unknown sample is 0.75, calculate the protein concentration in the sample. (1.13mg/mL)

5. You are preparing a standard curve for DNA quantification using spectrophotometry. The absorbance of your DNA standards at 260 nm is as follows:

Concentration (µg/mL)	Absorbance (260 nm)
10	0.21
20	0.41
30	0.63
40	0.81

Using the data as above,

a) Plot a standard curve, calculate the slope of the standard curve.
b) Calculate the DNA concentration (in µg/mL) in an unknown sample with an absorbance of 0.52. (26µg/mL)

6. Spectrophotometry relies on the Beer-Lambert law, which states that absorbance is proportional to the concentration of a substance. What limitations or assumptions in this law could lead to inaccuracies in experimental results when measuring absorbance in biological samples?

The Beer-Lambert law assumes that:

- The solution is homogeneous and there is no scattering of light.
- The solute behaves as a perfect absorber at a specific wavelength.
- There are no interactions between solute molecules that might affect absorbance (e.g., aggregation or dissociation).