BIO 107 Lab # 3: Summary & Study guide

You conducted a Biuret Protein Assay to determine the concentration of BSA (bovine serum albumin) in 4 unknown samples. In order to do this, you did the following:

Part I: Standard curve with BSA samples of known concentration
- You used the spectrophotometer and the Biuret reaction with BSA to measure the absorbance of 5 samples of BSA at 550 nm
  - 550 nm is the wavelength at which Biuret has maximum absorbance
- The known BSA samples had increasing concentrations from 1 mg/ml to 5mg/ml
- You calibrated the spectrophotometer using a blank that contained Biuret but not BSA to cancel out any absorbance by Biuret (any absorbance not resulting from BSA)
- The Beer-Lambert law applied: the higher the protein concentration, the more absorbance was measured (due to more intense color of Biuret)
- Data were recorded (table 3) to plot a standard curve that follows the line equation: \( y = m x + b \)
- You determined the slope “m” from your standard curve

Part II: BSA Assay to determine the concentration of 4 unknown samples
- You used the spectrophotometer and the Biuret reaction with BSA to measure the absorbance of 4 unknown samples (A, B, C and D) of BSA at 550 nm
- You calibrated the spectrophotometer using a blank that contained Biuret, but not BSA to cancel out any absorbance by Biuret (any absorbance not resulting from BSA)
- The Beer-Lambert law applied again
- The absorbance values of the 4 unknown samples were placed on the y axis of your graph
- You interpolated to determine the concentration of each unknown sample from the graph directly
  - This was done by drawing a straight line between the y value and your standard curve (line graph), then connecting this intercept with another vertical straight line to the x-axis (see PowerPoint slide)
- You used the slope “m” determined from your standard curve to solve the line
equations for x
  - Y = mx + b; if b = 0 (your line intercepts the y axis in 0), then you can solve
    this simple equation y = mx to determine the value of x for each unknown.
  - If y = mx, then x = y/m. You used your slope “m” and the absorbance values
    for the 4 unknown samples to determine x = concentration in mg/ml for each
    unknown.
- You calculated the % error using the values in table 4, the actual concentration of
  the 4 samples provided by me and the % error equation (p. 35).
- The % error allows you to determine how reliable your data is.

Examples:

1) If your absorbance values for the standard curve were not reliable, then the value “m”
will be off, even if you use the correct slope calculations. This will affect the calculated
concentration for samples A – D, so it will not be close to the actual values provided by
me. Check your values.

2) If the absorbance values you measured for the 4 unknown samples were not reliable,
then the interpolated value of “x” will be off, even if you use the standard curve correctly
to determine x. This will affect the concentrations for samples A – D as determined from
the graph, so they will not be close to the actual values provided by me. Check your
values.

3) How reliable were your values? If there was some error, what is its likely
source?

Summary:
- The concentration of the 4 samples was determined 2x: from the standard curve
directly and from the line equation of your standard curve.

Study Guidelines:
- Review the concepts and methods involved in this lab using the lab manual and the
  PowerPoint presentation.
- Review this document to summarize and check your understanding
- Reflect on your own data