BL4820 Biochemistry Techniques

Information on Units and Standard Curve

UNITS OF AMOUNTS AND CONCENTRATION:

Amounts are always given in moles, mmoles, µmoles, nmoles, etc. (symbol can be shortened to "mol" -- for example µmol). You can not write 10^{-3} µmol because this is nmol. You can always write 10^{-3} mol, but there is no need to since mmol is simpler and a better way to represent your data. Please refrain from using exponentials for amounts. When the molecular weight of a substance is unknown (i.e. for a mixture of proteins), the amount can be given as the mass -- g, mg, µg, ng, etc.

Concentration is always given in M, mM, µM, nM, etc. (symbol "M" means moles per liter and so "µM" means micromoles per liter). You can not write 10-3 µM because this is nM. You can always write 10-3 M, but there is no need to since mM is simpler and a better way to represent your data. Please refrain from using exponentials for concentrations. For substances of unknown molecular weight (i.e. for a mixture of proteins), the concentration can be given as g/liter, mg/ml, µg/ml, ng/µl, etc.

If you need to calculate an amount from a volume and concentration:

vol x conc = amount.

For example, (60 µM) x (1.0 ml) = 60 nmol or 0.06 µmol.

An easy way to think about this is: 60 µM = 60 nmol/ml so 1 ml of it contains 60 nmol.

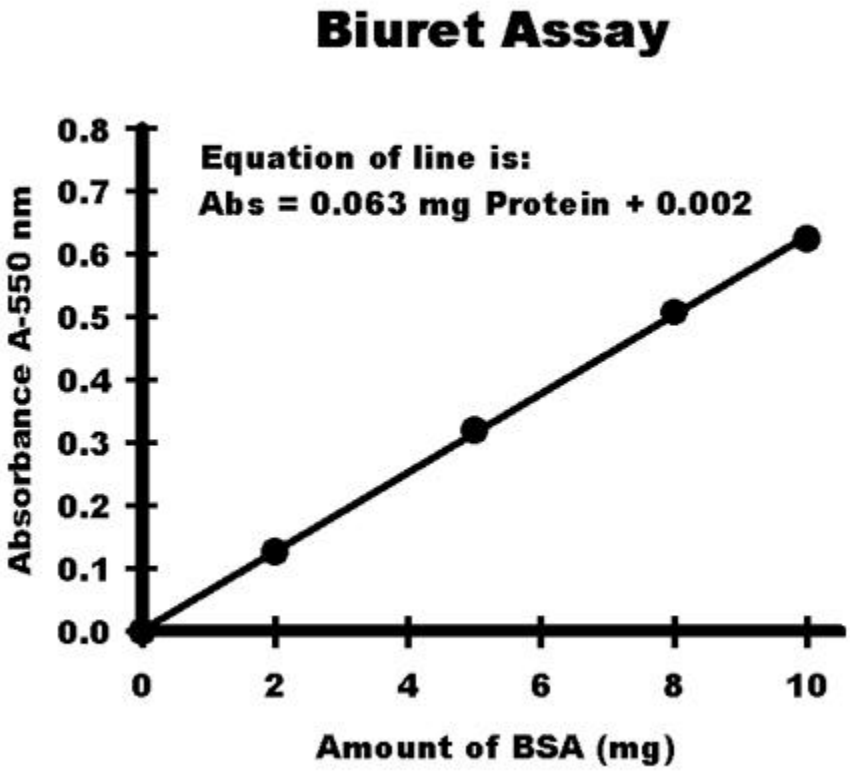
If you need to derive a concentration when you mix a fixed volume of a given concentration with another volume, use the final volume you arrive at to calculate the final concentration:

Final Concentration = (Initial Concentration X Initial Volume)/Final Volume

C2 = (C1 x V1)/V2

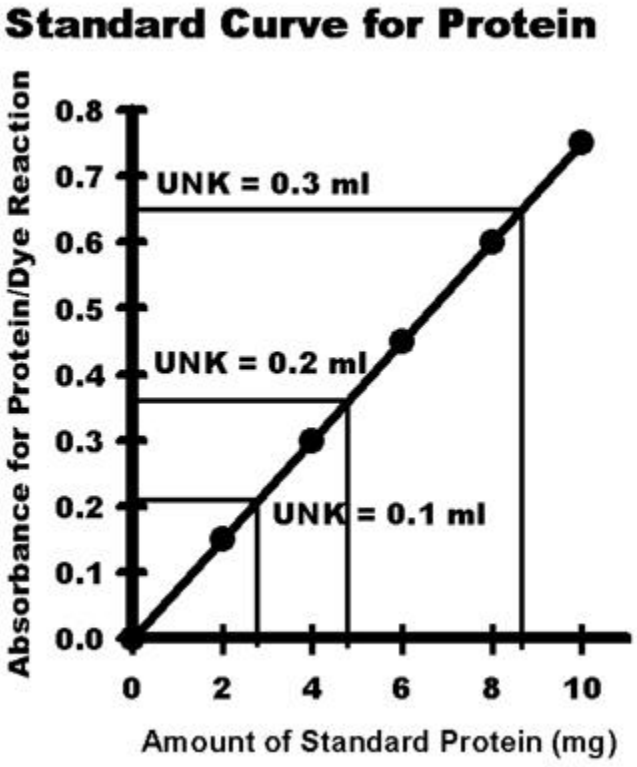
Standard Curves:

Standard Curves are used for quantitative analysis of unknown substances by comparison to a "standard".



For example, in evaluating the protein concentration of a sample, the amount in a given volume of unknown protein solution can be determined by comparison to the amounts of a "standard protein" using a dye that reacts with both the standard protein and the unknown. So determining the concentration of protein in the unknown solution is a two step process: 1) find out how much protein is present in fixed volumes of the unknown using the standard curve prepared with known amounts of the standard protein; and 2) calculate the concentration of protein in the unknown by dividing the protein amount by the volume of unknown used.

This concept is illustrated below:



Calculations for the Unknown Protein in this example:

Volume of Unknown (ml)	Absorbance	Amount of Protein (mg) from Standard Curve	Concentration (mg/ml)
0.01	0.011	Too Low to be Useful	----
0.1	0.210	2.8	28
0.2	0.360	4.8	24
0.3	0.650	8.6	29
0.5	0.910	Too High (Off Curve)	----

- The Volume of the Unknown (ml) is "given" information from the protocol instructions

in the text.
Absorbance is what you determined in the experiment in the lab.

- Amount of Protein (mg) is determined from the standard curve as shown by the lines drawn for specific volumes of unknown in the example standard curve shown above.

- Concentration is calculated by dividing the Amount of Protein (mg) by the Volume of Sample used to obtain that amount of unknown protein (ml) and the units of concentration are mg/ml.

- Once all the valid concentrations are calculated, then the average is taken. In this example,
  average = (28 + 24 + 29)/3 = 27 mg/ml (Remember to use the appropriate number of significant figures)

NOTE:

You can also calculate the Amount of Protein (mg) in the samples using the Equation of the Line for the Standard Curve:

Equation of Line = Linear Regression - a statistical method for calculating the equation of a line usually done on a calculator or computer.
Amount of Unknown Protein in mg = (Absorbance - y intercept)/slope; where the slope is expressed as absorbance/mg protein
(note this is the inversion of the normal result obtained for the graph where y = a + bx,
when y = absorbance, a = y intercept, and b = slope of line in terms of absorbance/amount of protein in mg).

For this example, equation of line = Amount of Unknown Protein (mg) = (specific Absorbance - 0.000)/0.075
For sample volume = 0.1 ml, Absorbance = 0.21; therefore
Amount of Unknown Protein (mg) = (0.210 - 0.000)/0.075 = 2.8 mg

Now using the volume of this sample, the concentration = 2.8 mg/0.1 ml = 28 mg/ml

Note: Not all standard curves go through the origin, which means that the Y-intercept may be a significant value in making your calculation of the amount of protein.