Worksheet 11: Spectrophotometry

1. Transferrin is the iron-transport protein found in blood. It has a molecular mass of 81 000 and carries two Fe^{3+} ions. Desferrioxamine B is a chelator used to treat patients with iron overload. It has a molecular mass of about 650 and can bind one Fe^{3+}. Desferrioxamine can take iron from many sites within the body and is ecreted through the kidneys. Molar absorptivities of these compounds at two wavelengths are given in the table. Both compounds are colorless in the absence of iron.

λ (nm)	Transferrin (M^{-1} cm^{-1})	Desferrioxamine (M^{-1} cm^{-1})
428	3540	2730
470	4170	2290

   (a) A solution of transferrin exhibits an absorbance of 0.463 at 470 nm in a 1.000-cm cell. Calculate the concentration of transferrin in milligrams per milliliter.

   (b) After adding desferrioxamine (which dilutes the sample), the absorbance at 470 nm was 0.424 and the absorbance at 428 nm was 0.401. Calculate the concentrations of both components.

2. For each of the definitions below, write the appropriate term in the blank.

   (a) _____ - A blank sample containing all components except for analyte that has not been subjected to the steps of the chemical analysis.

   (b) _____ - The concentration interval over which there is measurable response to a change in analyte concentration.

   (c) _____ - The quantities reported upon completion of statistical analysis of the data.

   (d) _____ - A blank sample exposed to the environment at the sample collection site and transported in the same manner as other samples back to the lab.

   (e) _____ - Individual measurements

   (f) _____ - The concentration interval over which the change in detector response is proportional to the analyte quantity.

   (g) _____ - The concentration interval over which linearity, accuracy and precision meet specifications.

   (h) _____ - A blank sample containing all components except analyte that has been carried through all steps of the chemical analysis, including sample preparation.

(i) _____ - The amounts or concentrations determined by using a calibration method.

3. In spectrophotometry, we measure the concentration of analyte by its absorbance of light. A low-concentration sample was prepared and nine replicate measurements gave absorbances of 0.0047, 0.0054, 0.0062, 0.0060, 0.0046, 0.0056, 0.0052, 0.0044, and 0.0058. Nine reagent blanks gave values of 0.0006, 0.0012, 0.0022, 0.0005, 0.0016, 0.0008, 0.0017, 0.0010, and 0.0011.

   (a) Find the absorbance detection limit using the equation below.

$$y_{\text{dl}} = y_{\text{blank}} + 3s$$

   (b) The calibration curve has a slope of 2.24×10^4 M^{-1}. Find the concentration detection limit with the equation below.

$$\text{Minimum detectable concentration} = \frac{3s}{m}$$

   (c) Find the lower limit of quantitation using the equation below.

$$\text{Lower limit of quantitation} = \frac{10s}{m}$$

4. Using the control chart below, identify each of the lines and determine whether or not the process being measured is under control.