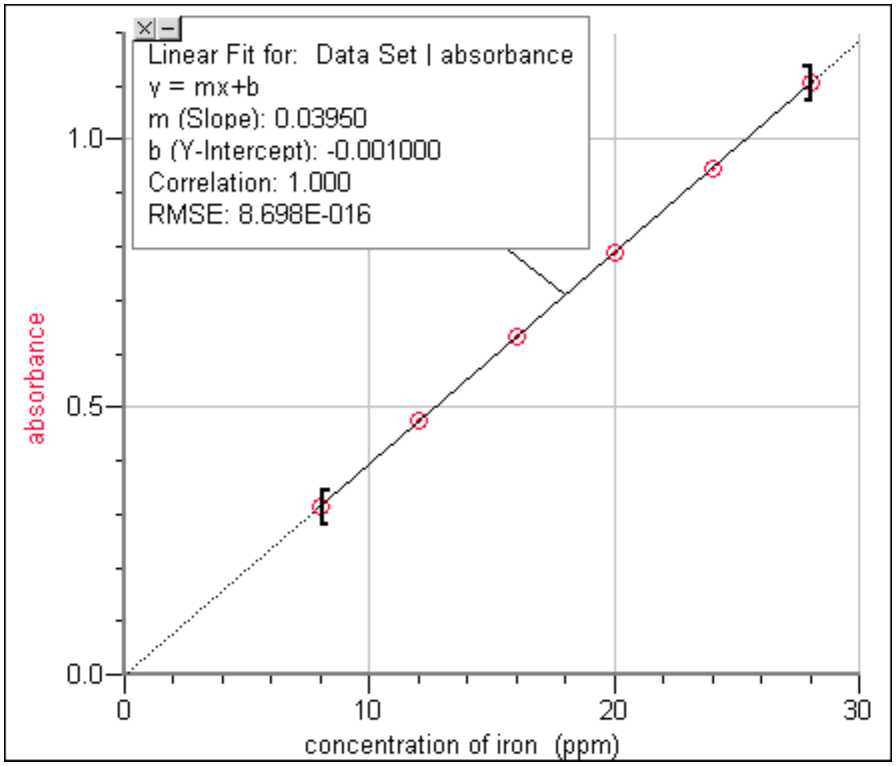
Answer Key to In-Class Quantitative Spectrophotometry Problems

1. A calibration curve is shown here:



Note that the concentrations of the standards have not been adjusted for dilution. Since all standards and the sample are treated identically (25 mL diluted to 50 mL) there is no need to correct for the dilution. The calibration equation is:

$$\text{Abs} = -0.0010 + 0.03950 \times [\text{Fe}^{2+}]$$

With an absorbance of 0.675, the concentration of Fe^{2+} in the sample is

$$0.675 = -0.0010 + 0.03950 \times [\text{Fe}^{2+}]$$

$$0.676 = 0.03950 \times [\text{Fe}^{2+}]$$

$$[\text{Fe}^{2+}] = 17.11 \text{ ppm}$$

2. For each analyte we can calculate the value of εb at each wavelength by dividing the absorbance by the concentration; thus:

$$\varepsilon b_{\text{NADH, 260 nm}} = (0.683/56.0 \text{ mM}) = 1.220 \times 10^{-2} \text{ mM}^{-1}$$

$$\varepsilon b_{\text{NADH, 340 nm}} = (0.0.285/56.0 \text{ mM}) = 5.089 \times 10^{-3} \text{ mM}^{-1}$$

$$\varepsilon b_{\text{CMP, 260 nm}} = (0.647/56.0\text{ mM}) = 1.155\times10^{-2}\text{ mM}^{-1}$$

$$\varepsilon b_{\text{CMP, 340 nm}} = (0.000/56.0\text{ mM}) = 0.00\text{ mM}^{-1}$$

For the sample, the absorbance at 340 nm is due solely to NADH; thus

$$0.371 = 5.089\times10\text{-}3\text{ mM}^{-1} \times [\text{NADH}]$$

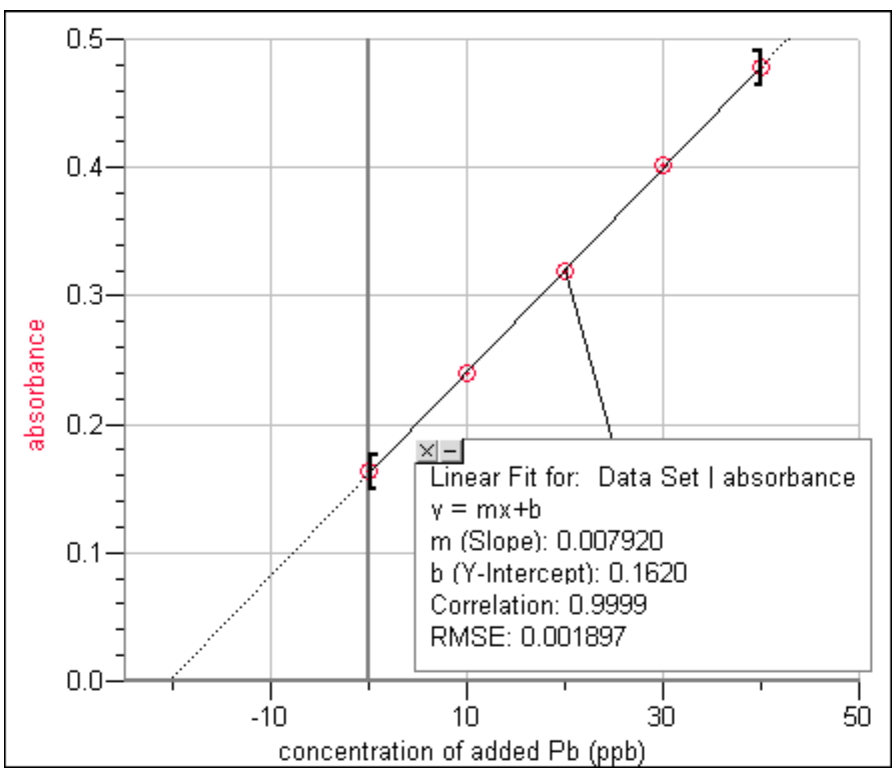
$$[\text{NADH}] = 72.9\text{ mM}$$

At a wavelength of 260 nm both NADH and CMP absorb; thus

$$1.50 = 1.220\times10^{-2}\text{ mM}^{-1}\text{ NADH} \times 72.9\text{ mM NADH} + 1.155\times10^{-2}\text{ mM}^{-1}\text{ CMP} \times [\text{CMP}]$$

$$[\text{CMP}] = 52.9\text{ mM}$$

3. With a standard addition we first need to determine the concentration of Pb^{2+} added to each solution. Diluting 0, 1, 2, 3, and 4 mL of the 1000 ppb standard to 100 mL gives concentrations of 0.00, 10.00, 20.00, 30.00 and 40.00 ppb Pb^{2+}. A plot of absorbance vs. the concentration of added Pb^{2+} is shown here:



The calibration equation for the standard additions is

$$\text{Abs} = 0.1620 + 0.007920 \times [Pb^{2+}]_{\text{added}}$$

The concentration of Pb^{2+} in the diluted sample is the absolute value of the *x*-intercept (where the absorbance is zero); thus

$$0.00 = 0.1620 + 0.007920 \times [Pb^{2+}]$$

$$[Pb^{2+}] = 20.45 \text{ ppb}$$

To find the concentration of Pb^{2+} in the original sample we account for its dilution of 10 mL sample to 100 mL total volume; thus, the concentration of Pb^{2+} in the original sample is 204.5 ppb.