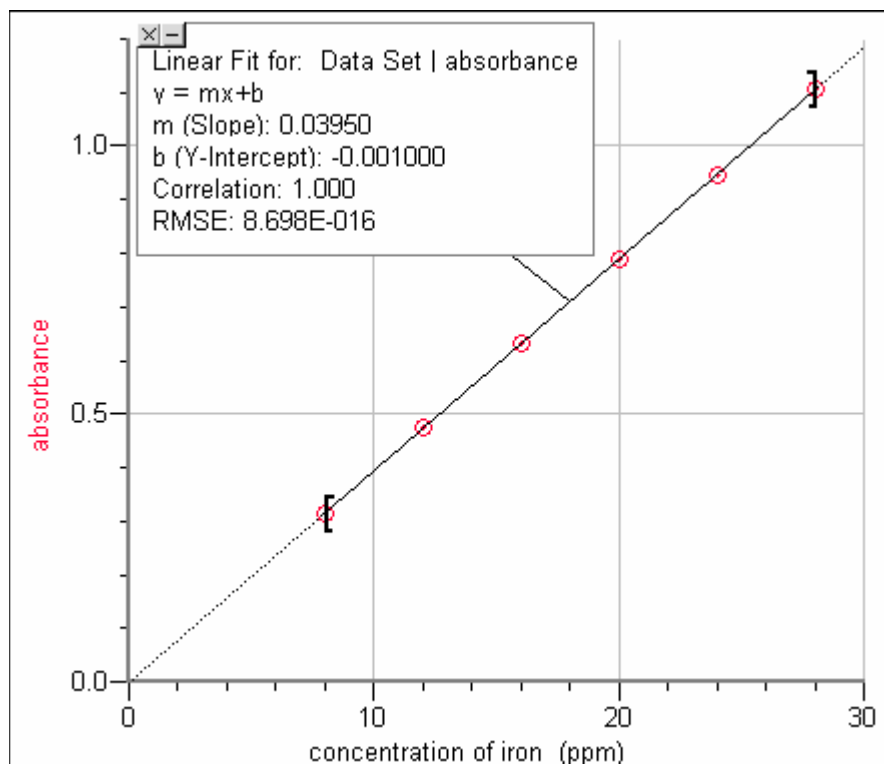


Answer Key to In-Class Quantitative Spectrophotometric Problems

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:

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

$$\epsilon b_{\text{NADH}, 340 \text{ nm}} = (0.0285/56.0 \text{ mM}) = 5.089 \times 10^{-3} \text{ mM}^{-1}$$

$$\epsilon b_{\text{CMP}, 260 \text{ nm}} = (0.647/56.0 \text{ mM}) = 1.155 \times 10^{-2} \text{ mM}^{-1}$$

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

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

$$0.371 = 5.089 \times 10^{-3} \text{ mM}^{-1} \times [\text{NADH}]$$

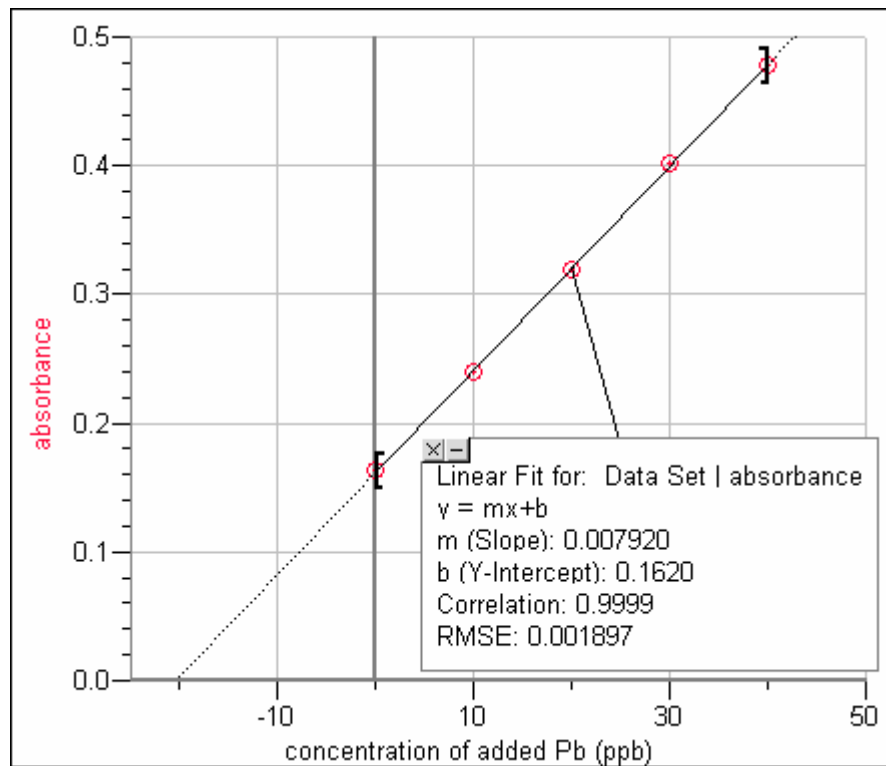
$$[\text{NADH}] = 72.9 \text{ mM}$$

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

$$1.50 = 1.220 \times 10^{-2} \text{ mM}^{-1} \text{ NADH} \times 72.9 \text{ mM NADH} + 1.155 \times 10^{-2} \text{ mM}^{-1} \text{ CMP} \times [\text{CMP}]$$

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

- With a standard addition we need to first 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

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

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

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

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

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.