

Quantitative Chemical Analysis

Seventh Edition



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To find the quantity of protein, substitute the measured absorbance into Equation A:

$$0.375 = -1.17 \times 10^{-4}x^2 + 0.01858x - 0.0007$$

This equation can be rearranged to

$$1.17 \times 10^{-4}x^2 - 0.01858x + 0.3757 = 0$$

Substituting $a = 1.17 \times 10^{-4}$, $b = -0.01858$, and $c = 0.3757$ into these equations gives

$$x = 135 \mu\text{g} \quad x = 23.8 \mu\text{g}$$

Figure 4-11 tells us that the correct choice is 23.8 μg , not 135 μg .

linear region ($>20 \mu\text{g}$) in Figure 4-11. The dashed curve that goes up to 25 μg of protein comes from a least-squares fit of the data to the equation $y = ax^2 + bx + c$ (Box 4-2).

The **linear range** of an analytical method is the analyte concentration range over which response is proportional to concentration. A related quantity defined in Figure 4-12 is **dynamic range**—the concentration range over which there is a measurable response to analyte, even if the response is not linear.

Before using your calculator or computer to find the least-squares straight line, make a graph of your data. The graph gives you an opportunity to reject bad data or stimulus to repeat a measurement or to decide that a straight line is not an appropriate function. *Examine your data for sensibility.*

It is not reliable to extrapolate any calibration curve, linear or nonlinear, beyond the measured range of standards. Measure standards in the entire concentration range of interest.

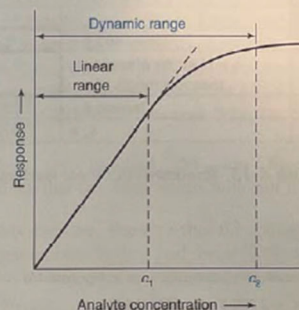


Figure 4-12 Calibration curve illustrating linear and dynamic ranges.

Propagation of Uncertainty with a Calibration Curve

In the preceding example, an unknown with a corrected absorbance of $y = 0.302$ had a protein content of $x = 18.2_4 \mu\text{g}$. What is the uncertainty in the number 18.2₄? A full treatment of the propagation of uncertainty gives the following results:^{1,9}

$$\text{Uncertainty in } x (= s_x) = \frac{s_y}{|m|} \sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{m^2 \sum (x_i - \bar{x})^2}} \quad (4-27)$$

where s_y is the standard deviation of y (Equation 4-20), $|m|$ is the absolute value of the slope, k is the number of replicate measurements of the unknown, n is the number of data points for the calibration line (14 in Table 4-7), $\bar{y}$ is the mean value of y for the points on the calibration line, x_i are the individual values of x for the points on the calibration line, and $\bar{x}$ is the mean value of x for the points on the calibration line. For a single measurement of the unknown, $k = 1$ and Equation 4-27 gives $s_x = \pm 0.3_9 \mu\text{g}$. If you measure four replicate unknowns ($k = 4$) and the average corrected absorbance is 0.302, the uncertainty is reduced from $\pm 0.3_9$ to $\pm 0.2_3 \mu\text{g}$.

The confidence interval for x is $\pm ts_x$, where t is Student's t (Table 4-2) for $n - 2$ degrees of freedom. If $s_x = 0.2_3 \mu\text{g}$ and $n = 14$ points (12 degrees of freedom), the 95% confidence interval for x is $\pm (2.179)(0.2_3) = \pm 0.5_0 \mu\text{g}$.

y = absorbance of unknown = 0.302
 x_i = μg of protein in standards in Table 4-7
 = (0, 0, 0, 5.0, 5.0, 5.0, 10.0, 10.0, 10.0, 15.0, 15.0, 20.0, 20.0, 20.0)
 $\bar{y}$ = average of 14 y values = 0.161₈
 $\bar{x}$ = average of 14 x values = 9.64₃ μg

To find values of t that are not in Table 4-2, use the Excel function TINV. For 12 degrees of freedom and 95% confidence, the function TINV(0.05,12) returns $t = 2.179$.

4-9 A Spreadsheet for Least Squares

Figure 4-13 implements least-squares analysis, including propagation of error with Equation 4-27. Enter values of x and y in columns B and C. Then select cells B10:C12. Enter the formula = LINEST(C4:C7,B4:B7,TRUE,TRUE) and press CONTROL+SHIFT+ENTER on a PC or COMMAND+RETURN on a Mac. LINEST returns m , b , s_m , s_b , R^2 , and s_y in cells B10:C12. Write labels in cells A10:A12 and D10:D12 so you know what the numbers in cells B10:C12 mean.

Cell B14 gives the number of data points with the formula = COUNT(B4:B7). Cell