Lecture 4

Statistics: Calibration Curves, Linear Regression.

Reading: Ch. 4
Outline

1. Calibration graphs in instrumental analysis (4-8).

2. The method of least squares (4-7).
Calibration graphs in instrumental analysis (4-8).

Concentration (x) Signal (y)

\[
\begin{align*}
  x_1 & \quad y_1 \\
  x_2 & \quad y_2 \\
  \cdot & \quad \cdot \\
  \cdot & \quad \\
  x_n & \quad y_n
\end{align*}
\]
Calibration graphs in instrumental analysis (4-8).

<table>
<thead>
<tr>
<th>Concentration (x)</th>
<th>Signal (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$x_1$</td>
<td>$y_1$</td>
</tr>
<tr>
<td>$x_2$</td>
<td>$y_2$</td>
</tr>
<tr>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>$x_n$</td>
<td>$y_n$</td>
</tr>
</tbody>
</table>

The product-moment correlation coefficient

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}}$$

graphs:

- $r = 1$
- $r = 0$
- $r = -1$
Calibration graphs in instrumental analysis (4-8).

<table>
<thead>
<tr>
<th>Amount of protein (μg)</th>
<th>Absorbance of independent samples</th>
<th>Range</th>
<th>Corrected absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.099 0.099 0.100</td>
<td>0.001</td>
<td>$-0.003_3$ $-0.003_3$ $0.000_7$</td>
</tr>
<tr>
<td>5.0</td>
<td>0.185 0.187 0.188</td>
<td>0.003</td>
<td>$0.085_7$ $0.087_7$ $0.088_7$</td>
</tr>
<tr>
<td>10.0</td>
<td>0.282 0.272 0.272</td>
<td>0.010</td>
<td>$0.182_7$ $0.172_7$ $0.172_7$</td>
</tr>
<tr>
<td>15.0</td>
<td>0.345 0.347 (0.392)</td>
<td>0.047</td>
<td>$0.245_7$ $0.247_7$ —</td>
</tr>
<tr>
<td>20.0</td>
<td>0.425 0.425 0.430</td>
<td>0.005</td>
<td>$0.325_7$ $0.325_7$ $0.330_7$</td>
</tr>
<tr>
<td>25.0</td>
<td>0.483 0.488 0.496</td>
<td>0.013</td>
<td>$0.383_7$ $0.388_7$ $0.396_7$</td>
</tr>
</tbody>
</table>

Table 4-7  Spectrophotometer data used to construct calibration curve

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Calibration graphs in instrumental analysis (4-8).

![Graph showing calibration data points and absorbance versus protein analyzed.](image)
Calibration graphs in instrumental analysis (4-8).

Figure 4-12
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Calibration graphs in instrumental analysis (4-8).

How to construct a calibration curve

1. Prepare known samples of analyte covering a range of concentrations expected

2. Run the measurement of each of calibration samples.

3. Make a graph of an analytical signal versus concentration.

Questions to answer

1. Is the calibration graph linear?

2. If it is a curve, what is the form of the curve?

3. Assuming that each point on the calibration curve is subject to error, what is the best straight line (or curve) through these points?
Figure 4-11
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The method of least squares (4-7)

Concentration (x) Signal (y)

\[
x_1 \quad y_1 \\
x_2 \quad y_2 \\
\vdots \quad \vdots \\
x_n \quad y_n
\]

\[y = mx + b\]

\[
m = \frac{n \sum (x_i y_i) - \sum x_i \sum y_i}{n \sum (x_i^2) - \left( \sum x_i \right)^2}
\]

\[
b = \frac{n \sum (x_i^2) \sum y_i - \sum (x_i y_i) \sum x_i}{n \sum (x_i^2) - \left( \sum x_i \right)^2}
\]
The method of least squares (4-7)

Concentration (x) Signal (y)

\[
\begin{align*}
x_1 & \quad y_1 \\
x_2 & \quad y_2 \\
\vdots & \quad \vdots \\
x_n & \quad y_n
\end{align*}
\]

\[y = mx + b\]

\[d_i = y_i - y = y_i - (mx_i + b)\]

\[
\sigma_y \approx s_y = \sqrt{\frac{\sum (d_i)^2}{n-2}}
\]

\[
s_m = \sqrt{\frac{ns_y^2}{n\sum (x_i^2) - \left(\sum x_i\right)^2}}
\]

\[
s_b = \sqrt{\frac{s_y^2 \sum (x_i^2)}{n\sum (x_i^2) - \left(\sum x_i\right)^2}}
\]

\[
s_x = \frac{s_y}{|m|} \sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{m^2\sum (x_i - \bar{x})^2}}
\]