A First Course on Kinetics and Reaction Engineering

Example 9.4

Problem Purpose

This problem illustrates the use of a Lineweaver-Burk plot to determine the values of the constants in a Michaelis-Menten rate expression.

Problem Statement

Suppose the enzyme-catalyzed reaction (1) is believed to obey Michaelis-Menten kinetics, equation (2). To test this, the rate of production of P was measured as a function of the concentration of S using a 10 L chemostat and 100 mg of enzyme. The temperature, pressure and solution volume were all constant over the course of the experiments. On the basis of the resulting data, presented in Table 1, does equation (2) offer an acceptable description of the reaction rate? If so, what are the best values of V_{max} and K_m?

Table 1. Data for Example 9.4

C_S (M)	r_P (M/min)
0.1	0.0009206
0.0856	0.0009217
0.0796	0.0009225
0.0747	0.0009182
0.0695	0.0009204
0.0629	0.0009170
0.0555	0.0009173
0.0482	0.0009150
0.0470	0.0009169
0.0412	0.0009165
0.0364	0.0009144
0.0302	0.0009105
0.0247	0.0009044
0.0209	0.0009009
0.0151	0.0008911
0.0102	0.0008782

$$S \rightarrow P \tag{1}$$

$$r_P = \frac{V_{\max} C_S}{K_m + C_S} \tag{2}$$

Problem Analysis

This problem involves finding the parameters in a simple Michaelis-Menten rate expression using measured values of the rate versus the substrate concentration. Since there are more data points (16) than unknown kinetic parameters (2), this is best accomplished by least-squares fitting. A linearized form of the Michaelis-Menten kinetic expression can be generated by taking the reciprocal of that expression. Then, having a linearized model equation, linear least squares can be used to fit a straight line to the data and obtain the “best” values of the parameters appearing in the equation.

Problem Solution

As noted in the informational reading for this unit, taking the reciprocal of a simple Michaelis-Menten kinetic expression yields equation (3). Because V_{max} and K_m are constants, this equation has the form of a straight line, equation (4), if y and x are defined as shown in equations (5) and (6). That is, equation (3) is a linear model for the dependence of y on x. A plot of y vs. x in this case is called a Lineweaver-Burk plot.

$$\frac{1}{r} = \left(\frac{K_m}{V_{\max}}\right)\frac{1}{C_S} + \frac{1}{V_{\max}} \tag{3}$$

$$y = mx + b \tag{4}$$

$$y = \frac{1}{r} \tag{5}$$

$$x = \frac{1}{C_S} \tag{6}$$

In this problem, we are asked to test whether a mathematical model offers an accurate representation of experimental data. Problems of this type can be solved by fitting the model equation to the data and then assessing the quality of the fit statistically and visually. In this particular problem, the model equation is linear, so linear least squares can be used to fit it to the experimental data (see Supplemental Unit S3). Linear least squares fitting can be performed manually, using a calculator, using a spreadsheet or using mathematics software. No matter which tool one chooses to use, it will be necessary to provide the following information and input data:

- the number of independent (x) variables
- whether or not the model includes an intercept (b)

- a set of experimental data points, each of which consists of a value for the dependent variable (y) and corresponding values for each of the independent variables (x_i)

In the present case, there is one independent variable in the model (x), and the model does include the y-intercept, b. It is trivial to generate the set of x and y values to be used in the fitting process: simply take the reciprocal of each of the C_S and r_P values to calculate corresponding x and y values according to equations (5) and (6). When this information and input data are provided to whichever linear least squares fitting tool one chooses to employ, the resulting output shows that the correlation coefficient, r^2, is greater than 0.99, the best value of the slope, m, is equal to 0.63 ± 0.04 min and the best value of the y-intercept, b, is equal to 1080 ± 2 min M^{-1}. (Here the uncertainties are the 95% confidence limits, other least squares fitting tools might provide different measures of the uncertainties in the fitted parameter values.) In most cases, a model plot like that shown in Figure 1 is also provided, but if such a plot is not provided, one can be generated easily.

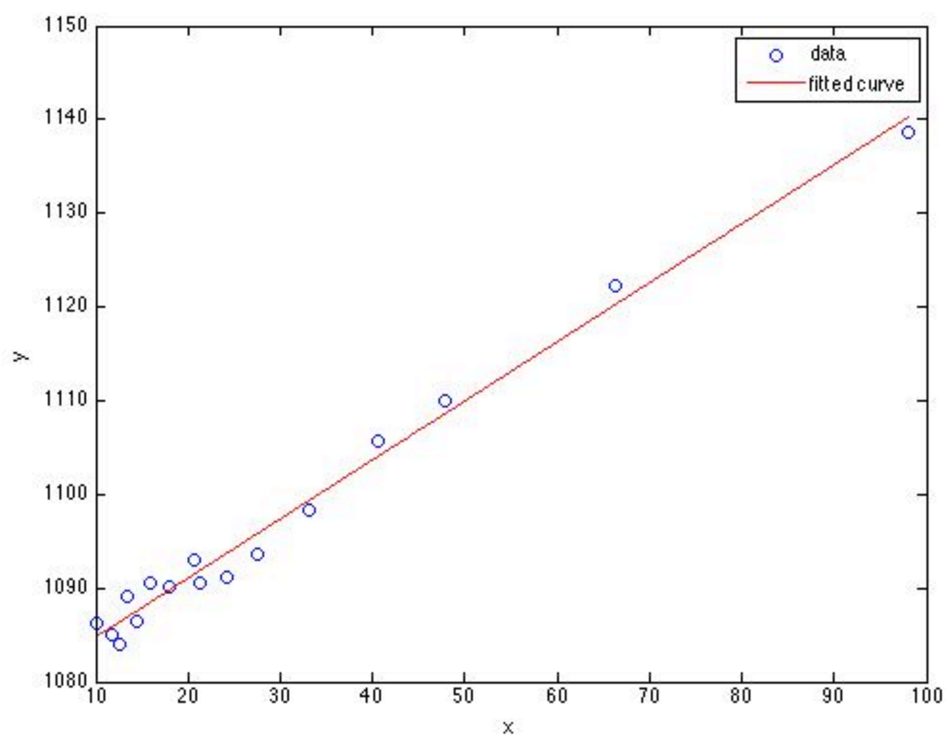

Figure 1. Model plot showing the experimental data as points and the model's predictions as a line.

Before the best values for m and b can be accepted, one must decide whether the final model is sufficiently accurate. In this case, the accuracy of the model can be assessed using the correlation coefficient and the model plot. The closer the correlation coefficient is to a value of 1.0, the better the fit of the model to the data. In this case, the correlation coefficient of 0.99 indicates a good fit. Additionally, if the fit is accurate, then the scatter of the experimental data about the model should be small and random; there should not be any systematic deviations of the data from the model. Examining the model plot, Figure 1, it is apparent that these criteria are also satisfied. Thus, the model does appear to be sufficiently accurate and the values of the slope and intercept can accepted, that is, the Michaelis-Menten rate expression does accurately predict the variation of the rate as a function of substrate concentration.

The problem asks for the best values of V_{max} and K_m. Comparing equation (4) to equation (3), and noting the definitions of x and y given in equations (5) and (6), it can be seen that V_{max} is related to the intercept according to equation (7) and K_m is related to both the slope and the intercept according to equation (8). A differential error analysis shows that if a model parameter, p, is related to the slope, m, and intercept, b, of a linearized from of the model, as in equation (9), then the uncertainty in that parameter, λ_p, is related to the slope, intercept and their uncertainties, λ_m and λ_b, according to equation (10). Applying that relationship to the present problem shows that the uncertainties in V_{max} and K_m should be calculated using equations (11) and (12).

$$b = \frac{1}{V_{\max}} \quad \Rightarrow \quad V_{\max} = \frac{1}{b} \tag{7}$$

$$m = \frac{K_m}{V_{\max}} = \quad \Rightarrow \quad K_m = mV_{\max} = \frac{m}{b} \tag{8}$$

$$p = f\left(m,b\right) \tag{9}$$

$$\lambda_p = \sqrt{\left(\frac{\partial f}{\partial m}\right)^2 \lambda_m^2 + \left(\frac{\partial f}{\partial b}\right)^2 \lambda_b^2} \tag{10}$$

$$\lambda_{V_{\max}} = \frac{\lambda_b}{b^2} \tag{11}$$

$$\lambda_{K_m} = \sqrt{\left(\frac{\lambda_m}{b}\right)^2 + \left(\frac{m\lambda_b}{b^2}\right)^2} \tag{12}$$

Thus, using the values of m, λ_m, b and λ_b resulting from fitting the linear form of the Michaelis-Menten kinetic expression to the data, the best values of the parameters can be calculated to be V_{max} = (9.27 ± 0.01) x 10^{-4} M min^{-1} and K_m = (5.84 ± 0.39) x 10^{-4} M.

Calculation Details Using MATLAB

Three MATLAB script files are provided with Supplemental Unit S3. The file names indicate the number of independent variables and whether or not the model has an intercept. The script named FitLinmSR is used when the model has one independent variable (x) and *does not* include the intercept

(b). FitLinmbSR is used when the model has one independent variable and *does* include the intercept, and FitLinSR is used when the model has two or more independent variables. (With MATLAB, when the model has two or more independent variables, it must have an intercept; Supplemental Unit S3 describes how to convert a model without an intercept into a model that has an intercept.) In this problem the model has one independent variable and an intercept, so the script file named FitLinmbSR.m will be used. To do so, the script file must be located in the current MATLAB working directory or in the MATLAB search path.

Before executing FitLinmbSR, the experimental values of x must be stored in a vector named `x`, and the experimental values of y must be stored in a vector named `y_hat`. Upon execution of the script, it will return the correlation coefficient as `r_squared`, the slope as `m`, the 95% confidence limits on the slope as `m_u`, the intercept as `b` and the 95% confidence limits on the intercept as `b_u`. It will also generate a model plot.

Therefore the following steps must be performed in order to solve this problem using MATLAB:

- enter the values of C_S as a vector (here I named it `CS`)
- enter the values of r_P as a vector (here I named it `rP`)
- calculate the values of x and store them in a vector named `x`
- calculate the values of y and store them in a vector named `y_hat`
- execute the MATLAB script file named FitLinmb
- use the returned values of `m`, `m_u`, `b` and `b_u` to calculate V_{max} (here I named it `Vmax`), K_m (here I named it `Km`), $\lambda_{V_{\max}}$ (here I named it `lambda_Vmax`) and $\lambda_{K_{\mathrm{m}}}$ (here I named it `lambda_Km`) according to equations (7) through (10)

The steps above could be performed at the MATLAB command prompt. Instead, I put them in a MATLAB script file named Example_9_4.m which accompanies this example. Listing 1 shows the contents of that file, except to save space only the entry of the first and last values of the vectors `CS` and `CP` is shown. The output generated upon execution of Example_9_4 in MATLAB is shown in Listing 2.

```
% MATLAB file used in the solution of Example 9.4 of "A First Course on
% Kinetics and Reaction Engineering."

% Enter the data from the table
CS = [0.1
      ⋮
0.0102];

rP = [0.0009206
      ⋮
0.0008782];

% Calculate the x and y_hat values
x = 1./CS;
y_hat = 1./rP;

% Use the MATLAB script file "FitLinmbSR.m" from "A First Course on
% Kinetics and Reaction Engineering" to fit a straight line with slope and
% intercept to the data
FitLinmbSR

% Calculate Vmax and its 95% confidence limits
Vmax = 1/b
lambda_Vmax = b_u/b^2

% Calculate Km and its 95% confidence limits
Km = m*Vmax
lambda_Km = sqrt(m_u^2/b^2 + (m*b_u/b^2)^2)
```

Listing 1. MATLAB script file Example_9_4 used in the solution of this problem.

```
>> Example_9_4

r_squared =
    0.9865


m =
    0.6303


m_u =
    0.0423


b =
   1.0784e+03


b_u =
    1.5904


Vmax =
   9.2727e-04


lambda_Vmax =
   1.3675e-06


Km =
   5.8441e-04


lambda_Km =
   3.9243e-05
```

Listing 2. MATLAB command window output upon running Example_9_4.m.