

A First Course on Kinetics and Reaction Engineering

Example 13.2

Problem Purpose

This example illustrates the analysis of kinetics data from a CSTR where the rate expression must be linearized.

Problem Statement

A new enzyme has been found for the dehydration reaction given in equation (1). A series of experiments were performed using a CSTR operating at steady-state and isothermally. The inlet flow rate was fixed at 5 mL per min and the reactor fluid volume was constant at 50 mL in all experiments. The inlet concentration of substrate, S, was changed for each experiment and the data given below for the product, P, concentration were recorded. Determine whether Michaelis-Menten kinetics adequately describe the rate of reaction, and if they do, determine the best values for the two kinetic parameters in the Michaelis-Menten rate equation.



<i>Inlet S Concentration (mmol/L)</i>	<i>Outlet P Concentration (mmol/L)</i>
12.6	1.01
11.2	0.98
9.0	0.92
8.1	0.90
6.3	0.83
5.6	0.79
4.3	0.71
3.6	0.65
2.3	0.52
1.0	0.29

Problem Analysis

In this problem we are given kinetics data for an enzyme-catalyzed reaction from a CSTR and asked to determine whether a Michaelis-Menten rate expression describes the kinetics of the reaction. The problem will be solved by substituting the rate expression into the CSTR design equation and fitting the resultant equation to the data. If the fit is good, then the Michaelis-Menten rate expression, with the parameter values found from the fit, is suitable as a rate expression for the reaction studied.

Problem Solution

Recall that the procedure for generating a rate expression involves (1) choosing a reactor to use in the laboratory, generating a design equation for the reactor and validating that design equation, (2) gathering experimental data, (3) picking a mathematical function to test as a rate expression, (4) substituting the chosen rate expression into the design equation and fitting the resulting design equation to the experimental data, (5) deciding whether the fit is adequate, and (6) if the fit is adequate, calculating the best values for the kinetics parameters and estimating their uncertainties or, if the fit is not adequate, repeating the process from step (3). Here we assume that experiments have been performed to ensure that the reactor does indeed behave as an ideal CSTR and to ensure that physical processes are not limiting the rate of reaction.

For an isothermal CSTR operating at steady state and with only one reaction taking place, the mole balance given in equation (2), written for $i =$ any reactant or product, is all that is needed to model the reactor. The Michaelis-Menten rate expression (see Unit 9) is given in equation (3), where the square brackets denote concentration. Writing equation (2) for the product, P, noting that there is no P in the feed ($\dot{n}_P^0 = 0$), and substituting the Michaelis-Menten rate expression gives equation (4).

$$\dot{n}_i - \dot{n}_i^0 = Vr_{i,j} \quad (2)$$

$$r_p = \frac{V_{\max} [S]}{K_m + [S]} \quad (3)$$

$$\dot{n}_p = V \frac{V_{\max} [S]}{K_m + [S]} \quad (4)$$

The next step is to fit equation (4) to the experimental data. This could be done using a non-linear least squares approach (see Unit 16 and Supplemental Unit S4). Here, however, it will be noted that if the reciprocal of equation (4) is written, equation (5), and rearranged to give equation (6), the resulting equation is linear. This is perhaps more easily seen if x , y , m and b are defined as in equations (7) through (10) and substituted into equation (6), leading to equation (11). Equation (11) is the equation for a straight line with a slope equal to m and an intercept equal to b .

$$\frac{1}{\dot{n}_p} = \frac{1}{V} \frac{K_m + [S]}{V_{\max} [S]} \quad (5)$$

$$\frac{V}{\dot{n}_p} = \frac{K_m}{V_{\max} [S]} + \frac{1}{V_{\max}} \quad (6)$$

$$x = \frac{1}{[S]} \quad (7)$$

$$y = \frac{V}{\dot{n}_P} \quad (8)$$

$$m = \left(\frac{K_m}{V_{\max}} \right) \quad (9)$$

$$b = \left(\frac{1}{V_{\max}} \right) \quad (10)$$

$$y = mx + b \quad (11)$$

Here, 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)

Thus, before the fitting can be performed, it is necessary to calculate values of x and y corresponding to each of the experimental data points given in the table in the problem statement. The concentration of S appearing in equation (7) is the outlet concentration whereas the table provides the inlet concentration. A mole table, or the definition of extent of reaction, can be used to derive the relationship between the molar flow rates of S and P, equation (12). Expressing the molar flow rates in terms of the volumetric flow rates and concentrations, rearranging and noting that for a liquid phase system such as this, the inlet and outlet volumetric flow rates are equal leads to equation (14), from which the value of x can be computed, equation (15).

$$\dot{n}_S = \dot{n}_S^0 - \dot{n}_P \quad (12)$$

$$\dot{V}[S] = \dot{V}^0[S]^0 - \dot{V}[P] \quad (13)$$

$$[S] = [S]^0 - [P] \quad (14)$$

$$x = \frac{1}{[S]} = \frac{1}{[S]^0 - [P]} \quad (15)$$

In order to calculate the corresponding values of y using equation (8), the outlet molar flow rate of P is needed, since the reactor volume (50 mL) is given in the problem statement. This is found simply

using the definition of concentration as expressed in equation (16), which can be substituted into equation (8) leading to equation (17) for the calculation of y .

$$\dot{n}_P = \dot{V}[P] \quad (16)$$

$$y = \frac{V}{\dot{V}[P]} \quad (17)$$

Thus, we can generate a set of (x,y) data using equations (15) and (16). The model has only one independent variable, x , and it *does* include an intercept. With this information and input data we have everything needed to fit the model to the data. Upon doing so, using whichever linear least squares fitting tool one chooses to employ, the resulting output shows that the correlation coefficient, r^2 , is equal to 0.9988, the best value of the slope, m , is equal to 18.5 ± 0.5 min and the best value of the y-intercept, b , is equal to $8.67 \pm 0.27 \times 10^3$ mL min mmol⁻¹ (95% confidence limits based upon the data given in the problem statement). The fitting tool may also produce a model plot like that shown in Figure 1, but if such a plot is not created, one can be generated easily.

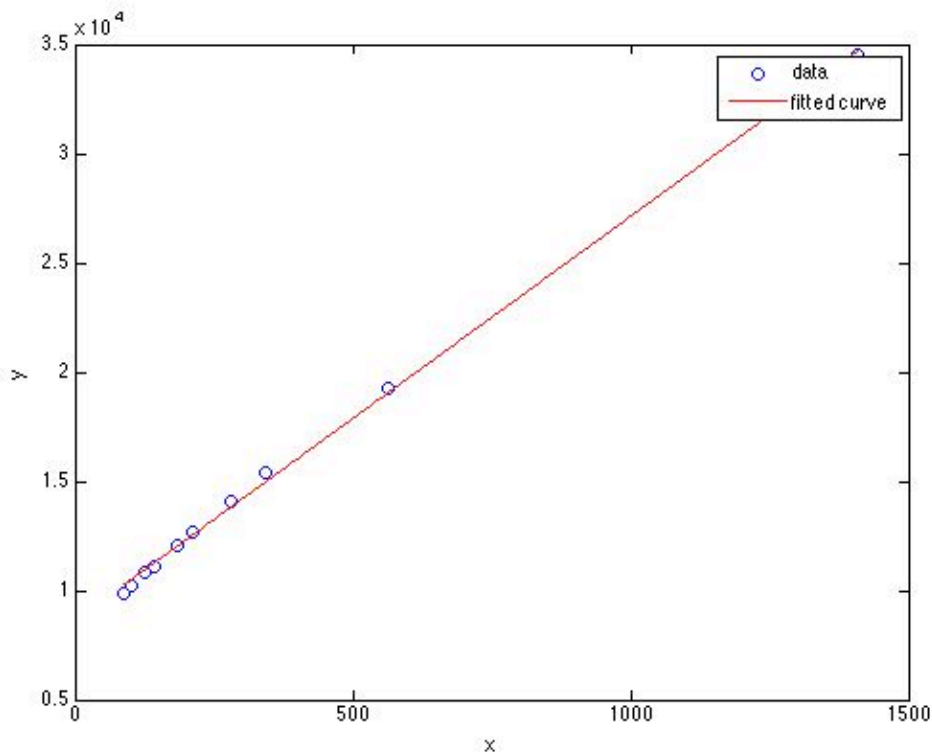


Figure 1. Model plot showing the experimental data as points and the model's predictions as a line.

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 0.9988 indicates an excellent 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 also are satisfied. Thus, the model does appear to be sufficiently accurate and the values of the slope and intercept can be accepted.

We are interested in the best values for the parameters, V_{max} and K_m , not the slope and intercept. The value of V_{max} can be found from the intercept, b , by rearranging equation (10) as shown in equation (18). Similarly, K_m can be found by rearranging equation (9), as shown in equation (19). A differential error analysis shows that if a model parameter, p , is related to the slope, m , and intercept, b , of a linearized form of the model, as in equation (20), 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 (21). Applying that relationship to the present problem shows that the uncertainties in V_{max} and K_m should be calculated using equations (22) and (23).

$$V_{\max} = \frac{1}{b} \quad (18)$$

$$K_m = mV_{\max} = \frac{m}{b} \quad (19)$$

$$p = f(m, b) \quad (20)$$

$$\lambda_p = \sqrt{\left(\frac{\partial f}{\partial m}\right)^2 \lambda_m^2 + \left(\frac{\partial f}{\partial b}\right)^2 \lambda_b^2} \quad (21)$$

$$\lambda_{V_{\max}} = \frac{\lambda_b}{b^2} \quad (22)$$

$$\lambda_{K_m} = \sqrt{\left(\frac{\lambda_m}{b}\right)^2 + \left(\frac{m\lambda_b}{b^2}\right)^2} \quad (23)$$

Applying equations (18), (19), (22) and (23) one finds that $V_{max} = 1.15 \pm 0.04 \times 10^{-4} \text{ mmol mL}^{-1} \text{ min}^{-1}$ and $K_m = 0.0021 \pm 0.00009 \text{ mmol mL}^{-1}$.

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 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, r^2 , as $r_squared$, the slope, m , as m , the 95% confidence limits on the slope, λ_m , as m_u , the intercept, b , as b and the 95% confidence limits on the intercept, λ_b , as b_u . It will also generate a model plot. Once these values are available, the best values for V_{max} and K_m and their uncertainties can be computed using equations (18), (19), (22) and (23). The commands for performing all these tasks can be entered at the MATLAB command prompt, but here they have been saved in the MATLAB file named Example_13_2.m which accompanies this solution. Listing 1 shows the code from Example_13_2.m and Listing 2 shows the output that it produces, except for the model plot.

```
% MATLAB file used in the solution of Example 13.2 of "A First Course on
% Kinetics and Reaction Engineering."

% Enter data provided in the problem statement in consistent units

V = 50; % mL
VFR = 5; % mL per min
CS0=[12.6 11.2 9.0 8.1 6.3 5.6 4.3 3.6 2.3 1.0]'; % mmol per L
CS0 = CS0/1000; % mmol per mL
CP=[1.01 0.98 0.92 0.90 0.83 0.79 0.71 0.65 0.52 0.29]'; % mmol per L
CP = CP/1000; % mmol per mL

% Calculate corresponding values of x and y_hat, equations (15) and (16)
x = 1./(CS0 - CP);
y_hat = V/VFR./CP;

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

% Calculate Vmax and Km and their uncertainties
Vmax = 1/b
Vmax_u = b_u/b^2
Km = m/b
Km_u = sqrt((m_u/b)^2 + (m*b_u/b^2)^2)
```

Listing 1. Contents of the file Example_13_2.m used to solve this problem using MATLAB.

```
>> Example_13_2
r_squared =
    0.9988
m =
    18.4636
m_u =
    0.5267
b =
    8.6732e+03
b_u =
    269.6153
Vmax =
    1.1530e-04
Vmax_u =
    3.5841e-06
Km =
    0.0021
Km_u =
    8.9819e-05
```

Listing 2. Output generated upon execution of Example_13_2.m.