Determination of the Michaelis-Menten kinetic parameters. (Problem 3.6 of Shuler and Kargi).
Instructor: Nam Sun Wang

$i := 0 .. 99$

$i := 0 .. \mathrm{last}(V)$

Enzyme Conc. (g/L) $E0_i :=$	Temperature (°C) $T_i :=$	Inhibitor Conc. (mmol/mL) $I_i :=$	Substrate Conc. (mmol/mL) $S_i :=$	Reaction Rate (mmole/mL·min) $V_i :=$
1.6	30	0	0.1	2.63
1.6	30	0	0.033	1.92
1.6	30	0	0.02	1.47
1.6	30	0	0.01	0.96
1.6	30	0	0.005	0.56
1.6	49.6	0	0.1	5.13
1.6	49.6	0	0.033	3.70
1.6	49.6	0	0.01	1.89
1.6	49.6	0	0.0067	1.43
1.6	49.6	0	0.005	1.11
0.92	30	0	0.1	1.64
0.92	30	0	0.02	0.90
0.92	30	0	0.01	0.58
0.92	30	0.6	0.1	1.33
0.92	30	0.6	0.033	0.80
0.92	30	0.6	0.02	0.57

The units for v_m, K_m , and K_I are:

$K_m = \frac{\mathrm{mmole}}{\mathrm{mL}}$ $\quad v_m = \frac{\mathrm{mmole}}{\mathrm{mL \cdot min}}$

$K_I = \frac{\mathrm{mmole}}{\mathrm{mL}}$

Case I. No inhibition, 30°C, E_0=1.6 g/L. $\quad v := \mathrm{submatrix}(V,0,4,0,0) \quad s := \mathrm{submatrix}(S,0,4,0,0)$

Fit data with the Lineweaver-Burk equation: $\frac{1}{v} = \frac{1}{v_m} + \frac{K_m}{v_m} \cdot \frac{1}{s}$

$v_m := \mathrm{intercept}\left(\overrightarrow{\frac{1}{s}}, \overrightarrow{\frac{1}{v}}\right)^{-1} \quad v_m = 3.295 \quad K_m := \mathrm{slope}\left(\overrightarrow{\frac{1}{s}}, \overrightarrow{\frac{1}{v}}\right) \cdot v_m \quad K_m = 0.0244$

Fit data with the Eadie-Hostee equation: $v = v_m - K_m \cdot \frac{v}{s}$

$v_m := \mathrm{intercept}\left(\overrightarrow{\frac{v}{s}}, v\right) \quad v_m = 3.286 \quad K_m := -\mathrm{slope}\left(\overrightarrow{\frac{v}{s}}, v\right) \quad K_m = 0.0243$

Fit data with the Hanes-Woolf equation: $\frac{s}{v} = \frac{K_m}{v_m} + \frac{1}{v_m} \cdot s$

$v_m := \mathrm{slope}\left(s, \overrightarrow{\frac{s}{v}}\right)^{-1} \quad v_m = 3.265 \quad K_m := \mathrm{intercept}\left(s, \overrightarrow{\frac{s}{v}}\right) \cdot v_m \quad K_m = 0.0240$

Nonlinear regression to minimize: $sse(v_m, K_m) := \sum \overrightarrow{\left(v - \overrightarrow{\frac{v_m \cdot s}{K_m + s}}\right)^2}$

$v_m := 1 \quad K_m := 0$ ... initial guess

Given $\quad sse(v_m, K_m) = 0 \quad 0 = 0 \quad \begin{pmatrix} v_m \\ K_m \end{pmatrix} := Minerr(v_m, K_m) \quad v_m = 3.269 \quad K_m = 0.0240$

Case II. No inhibition, 49.6°C, E_0=1.6 g/L. $\quad v := submatrix(V,5,9,0,0) \quad s := submatrix(S,5,9,0,0)$

Fit data with the Lineweaver-Burk equation: $\frac{1}{v} = \frac{1}{v_m} + \frac{K_m}{v_m} \cdot \frac{1}{s}$

$v_m := intercept\left(\overrightarrow{\frac{1}{s}}, \overrightarrow{\frac{1}{v}}\right)^{-1} \quad v_m = 6.343 \qquad K_m := slope\left(\overrightarrow{\frac{1}{s}}, \overrightarrow{\frac{1}{v}}\right) \cdot v_m \quad K_m = 0.0234$

Fit data with the Eadie-Hostee equation: $v = v_m - K_m \cdot \frac{v}{s}$

$v_m := intercept\left(\overrightarrow{\frac{v}{s}}, v\right) \quad v_m = 6.315 \qquad K_m := -slope\left(\overrightarrow{\frac{v}{s}}, v\right) \quad K_m = 0.0232$

Fit data with the Hanes-Woolf equation: $\frac{s}{v} = \frac{K_m}{v_m} + \frac{1}{v_m} \cdot s$

$v_m := slope\left(s, \overrightarrow{\frac{s}{v}}\right)^{-1} \quad v_m = 6.325 \qquad K_m := intercept\left(s, \overrightarrow{\frac{s}{v}}\right) \cdot v_m \quad K_m = 0.0233$

Nonlinear regression to minimize: $sse(v_m, K_m) := \sum \overrightarrow{\left(v - \overrightarrow{\frac{v_m \cdot s}{K_m + s}}\right)^2}$

$v_m := 1 \quad K_m := 0$ ... initial guess

Given $\quad sse(v_m, K_m) = 0 \quad 0 = 0 \quad \begin{pmatrix} v_m \\ K_m \end{pmatrix} := Minerr(v_m, K_m) \quad v_m = 6.323 \quad K_m = 0.0233$

Note: because K_m is a ratio of rate constants (whereas, v_m has one rate constant), K_m is less sensitive to temperature than v_m is.

Case IIIa. No inhibition, 30°C, E_0=0.92 g/L. $v := \text{submatrix}(V,10,12,0,0)$ $s := \text{submatrix}(S,10,12,0,0)$

Fit data with the Lineweaver-Burk equation: $\frac{1}{v} = \frac{1}{v_m} + \frac{K_m}{v_m} \cdot \frac{1}{s}$

$v_m := \text{intercept}\left(\overrightarrow{\frac{1}{s}}, \overrightarrow{\frac{1}{v}}\right)^{-1}$ $v_m = 2.048$ $K_m := \text{slope}\left(\overrightarrow{\frac{1}{s}}, \overrightarrow{\frac{1}{v}}\right) \cdot v_m$ $K_m = 0.0254$

Fit data with the Eadie-Hostee equation: $v = v_m - K_m \cdot \frac{v}{s}$

$v_m := \text{intercept}\left(\overrightarrow{\frac{v}{s}}, v\right)$ $v_m = 2.057$ $K_m := -\text{slope}\left(\overrightarrow{\frac{v}{s}}, v\right)$ $K_m = 0.0255$

Fit data with the Hanes-Woolf equation: $\frac{s}{v} = \frac{K_m}{v_m} + \frac{1}{v_m} \cdot s$

$v_m := \text{slope}\left(s, \overrightarrow{\frac{s}{v}}\right)^{-1}$ $v_m = 2.06$ $K_m := \text{intercept}\left(s, \overrightarrow{\frac{s}{v}}\right) \cdot v_m$ $K_m = 0.0256$

Case IIIb. /w inhibition, 30°C, E_0=0.92 g/L. $v := \text{submatrix}(V,13,15,0,0)$ $s := \text{submatrix}(S,13,15,0,0)$

Fit data with the Lineweaver-Burk equation: $\frac{1}{v} = \frac{1}{v_m} + \frac{K_m}{v_m} \cdot \frac{1}{s}$

$v_m := \text{intercept}\left(\overrightarrow{\frac{1}{s}}, \overrightarrow{\frac{1}{v}}\right)^{-1}$ $v_m = 2.009$ $K_{mapp} := \text{slope}\left(\overrightarrow{\frac{1}{s}}, \overrightarrow{\frac{1}{v}}\right) \cdot v_m$ $K_{mapp} = 0.0503$

Fit data with the Eadie-Hostee equation: $v = v_m - K_m \cdot \frac{v}{s}$

$v_m := \text{intercept}\left(\overrightarrow{\frac{v}{s}}, v\right)$ $v_m = 1.994$ $K_{mapp} := -\text{slope}\left(\overrightarrow{\frac{v}{s}}, v\right)$ $K_{mapp} = 0.0497$

Fit data with the Hanes-Woolf equation: $\frac{s}{v} = \frac{K_m}{v_m} + \frac{1}{v_m} \cdot s$

$v_m := \text{slope}\left(s, \overrightarrow{\frac{s}{v}}\right)^{-1}$ $v_m = 1.988$ $K_{mapp} := \text{intercept}\left(s, \overrightarrow{\frac{s}{v}}\right) \cdot v_m$ $K_{mapp} = 0.0494$

We compare Case IIIa and Case IIIb where the only difference is the presence of the inhibitor. $I := 0.6$
Since v_m does not depend on the presence of inhibitor but K_{mapp} does, the inhibitor is **competitive**.

$$K_{mapp} = K_m \cdot \left(1 + \frac{I}{K_I}\right) \longrightarrow K_I := \frac{K_m}{K_{mapp} - K_m} \cdot I \qquad K_I = 0.647$$