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Chemical Kinetics: Simple Enzyme Reactions

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Let S be a "substrate" material which is converted by the addition of an enzyme, E, into a molecular complex C which dissociates to yield a product P. We have:

$$S + E \underset{K_2}{\overset{K_1}{\rightleftharpoons}} C \overset{K_3}{\rightarrow} E + P.$$

This is, of course, an idealization of a more complex schema, since, in fact, even when S and P have the same molecular weight, energy must be used to, at least momentarily, change and restore the conformation of the enzyme E. But for many situations, our simple model will suffice.

Let $S(t)$, $E(t)$, $C(t)$, and $P(t)$ be the amounts of S, E, C, and P respectively at time t. Then we have:

$$\begin{aligned} dC/dt(t) &= K_1 S(t) E(t) - K_2 C(t) - K_3 C(t), \quad C(0) = 0, \\ dP/dt(t) &= K_3 C(t), \quad P(0) = 0, \\ S(t) &= S_0 - C(t) - P(t), \quad \text{with} \quad S_0 = S(0), \\ E(t) &= E_0 - C(t), \quad \text{with} \quad E_0 = E(0). \end{aligned}$$

Typically the reaction $C \rightarrow P + E$ is much slower than the complex-formation reaction $S + E \rightleftharpoons C$. Thus after the initial rise of C within time t_0, we have $dC/dt \approx 0$, so that then

$$\begin{aligned} C(t) &\approx K_1 S(t)(E0 - C(t))/(K2 + K3), \quad \text{or} \\ C(t) &\approx E_0 S(t)/[(K_2 + K_3)/K_1 + S(t)], \quad \text{for} \quad t > t_0. \end{aligned}$$

Thus, from the equation for dP/dt, we have the so-called Michealis-Menten equation:

$$dP/dt(t) \approx K_3 E_0 S(t)/((K_2 + K_3)/K_1 + S(t)), \quad \text{for} \quad t > t_0.$$

Now the slope $dP/dt(t)$ is measurable by fitting a straight-line to a segment of the kinetic curve for $P(t)$ with $t_0 < t \ll t_e$, where t_e is the time such that $S(t_e) \approx 0$, e.g., when almost all the substrate material has been consumed. Note $S(t) \approx S_0$ for $t_0 < t \ll t_e$ if S_0 is large enough, so one can fit the Michealis-Menten equation to the single data point $(S_0, dP/dt)$ to try to obtain K_3 and $K_m = (K_2 + K_3)/K_1$, the so-called Michealis-Menten constant.

The parameter K_3 may be independently resolved by observing that when S_0 is very large we have $S(t)/(K_m + S(t)) \approx 1$ for $t_0 < t \ll t_e$, so $dP/dt(t) \approx K_3 E_0$. Thus the maximum rate of formation of P for a fixed E_0 value is $K_3 E_0$. Let $K_3 E_0 = V_m$. V_m can be determined by measuring the slope, $dP/dt(t)$, of the linear region of the kinetic curve obtained when S_0 is very large. Now, the Michealis-Menten equation becomes:

$$dP/dt(t)/V_m \approx S(t)/(K_m + S(t)) \quad \text{for} \quad t_0 < t \ll t_e.$$

Note $dP/dt(t)/V_m$ is the relative rate of formation of P, i.e., the proportion of the maximum rate which is achieved. Hence, having measured V_m, one can then measure $dP/dt(t)$ for a relatively-large amount of S, and then obtain K_m from the Michealis-Menten equation as $S_0 V_m/(dP/dt(t)) - S_0$.

The Michealis-Menten constant is the amount of substrate which will yield a product formation rate of $V_m/2$. It thus is the point at which the formation of product becomes increasingly sensitive to a decreasing amount of substrate. The activity of the enzyme for a given amount of substrate is determined directly as $dP/dt(t)$ computed from the Michealis-Menten equation for a given K_m.

The Lineweaver-Burke form of the Michealis-Menten equation is often used because of its linear form. It is:

$$1/(dP/dt(t)) \approx (K_m/V_m)(1/S(t)) + (1/V_m).$$

The Eadie and Dixon form is also often used. It is:

$$S(t)/(dP/dt(t)) \approx S(t)/V_m + K_m/V_m.$$

Actually, as noted above, the reaction $S + E \rightleftharpoons C \rightarrow P + E$ is a fiction. It is commonly used to approximate the situation:

$$S + E \underset{K_2}{\overset{K_1}{\rightleftharpoons}} C \underset{K_4}{\overset{K_3}{\rightleftharpoons}} D \underset{K_6}{\overset{K_5}{\rightleftharpoons}} P + F$$

$$U + F \rightleftharpoons B \rightleftharpoons A \rightleftharpoons V + E$$

where C is ES-complex, and D is EP-complex and E and F are co-factors. Typically K_4 is negligible, but as the amount of P increases, it may block an appreciable amount of enzyme if K_6 is not nearly zero, and this can render the Michealis-Menten equation useless.

The following is an MLAB tutorial sequence for studying the Michaelis-Menten model relative to the simplified kinetic model.

First define the kinetic model by typing:

```
*FUNCTION C DIFF T(T) = K1*(S0-C-P)*(E0-C)-(K2+K3)*C
*FUNCTION P DIFF T(T) = K3*C
*INITIAL C(0)=0
*INITIAL P(0)=0
*S0 = 10; E0 = 1
*K1 = .2; K2 = .025; K3 = .025
```

Thus we have assumed the true situation is:

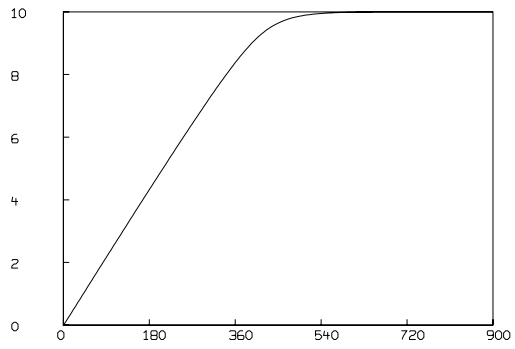
$$S + E \underset{.025}{\overset{.2}{\rightleftharpoons}} C \overset{.025}{\rightarrow} P + E$$

starting with 10 moles of substrate and 1 mole of enzyme. We may look at the kinetic behavior of this system over 900 seconds by typing:

```
*Q = INTEGRATE(P DIFF T, C DIFF T, 0:900:5)
*TYPE Q ROW 1:180:10
```

The first column of Q is time, t, the second is $P(t)$, the third is $dP/dt(t)$, the fourth is $C(t)$, and the fifth is $dC/dt(t)$. We can look at the graph of P vs. t by typing:

```
*DRAW Q COL 1:2, LINETYPE dashed
*VIEW
```

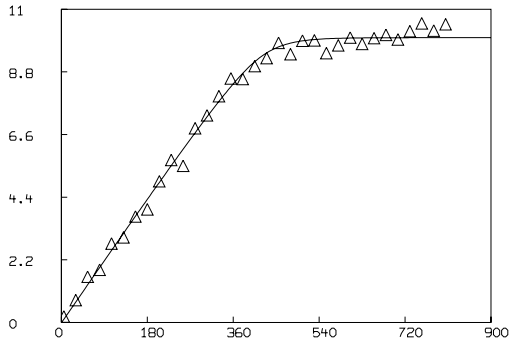


Now, let us generate some "laboratory data" about our reaction. We shall use the MLAB normal random number generator to generate normally distributed random numbers. Type:

```
*M = Q COL 1:2 ROW 2:162:5
*E = (NORMRAN ON 0^^NROWS(M))/4
*TYPE E
```

E is a vector of "normal" errors. Now type:

```
*M COL 2 = (M COL 2) + E
*DRAW M, POINTTYPE triangle LINETYPE none
*view
```

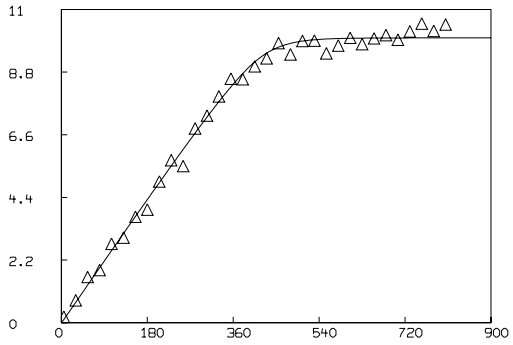


M is a matrix of P vs. t points "with error", as might have been measured in an actual laboratory situation. Now, let us "guess" K_1, K_2, and K_3 and try to determine them as functions of M. Type:

```
*K1 = .3; K2 = .01; K3 = .02
*CONSTRAINTS CX={K1>K2, K2>0, K3>0}
*METHOD = GEAR; ERRFAC = .002; MAXITER = 12
*FIT(K1,K2,K3), P to M, CONSTRAINTS CX
```

The control variables `METHOD` and `ERRFAC` are set based on prior experience; this problem is stiff and runs slowly! Our curve-fit "predicts" that K_1, K_2, and K_3 are 2.4295, .7975, and .025296 respectively, and resets them accordingly. Note K_1 and K_2 are not even close to .2 and .025, but K_3 is approximately correct. We may observe the graph of this fit by typing:

```
*Q1 = INTEGRATE(P DIFF T, C DIFF T, 0:900:5)
*DRAW Q1 COL 1:2 color green
*VIEW
```

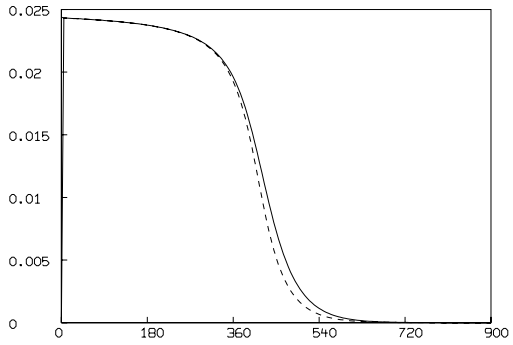


Q_1 is now a matrix of "kinetic concentration and velocity curves" for our reaction as determined by curve-fitting. Let us discard our picture by typing:

```
*DELETE W
```

Now, let us analyze the same reaction using the Michaelis-Menten model. Type:

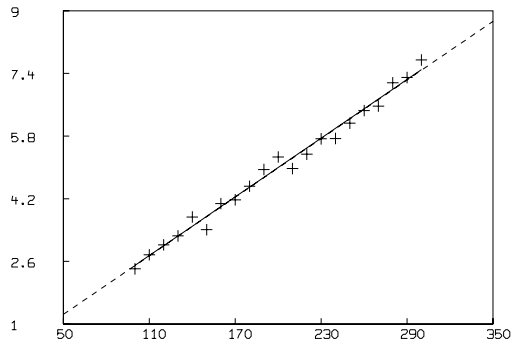
```
*K1 = .2; K2 = .025; K3 = K2
*FUNCTION MM(S)=VM*S/(KM+S)
*KM = (K2+K3)/K1; VM = K3*E0
*DRAW Q COL (1,3)
*SM = S0-(Q COL 2)-(Q COL 4)
*DRAW (Q COL 1)&'(MM ON SM), LINETYPE dashed
*view
```



Recall Q is the matrix of true curves corresponding to $K_1 = .2$, $K_2 = .025$, and $K_3 = .025$. The matrix SM is computed as the amounts of S at time $t = 0, 5, 10, \ldots, 900$. The curves we see are the rate of change, dP/dt vs. t and its Michaelis-Menten approximation.

Now, let us generate two runs of "laboratory" data using the error vector E and obtain the two constants V_m and K_m. Type:

```
*DELETE W
*S0 = 500
*Z = INTEGRATE (P DIFF T, C DIFF T, 100:300:10) COL 1:2
*DRAW Z
*Z COL 2 = (Z COL 2)+(NORMRAN ON 0^^NROWS(Z))/4
*DRAW Z, LINETYPE none, POINTTYPE crosspt
*FUNCTION Y(T)=A*T+B
*CONSTRAINTS QS = {A > 0, B > 0}
*A = 1; B = 1
*FIT(A,B), Y TO Z, CONSTRAINTS QS
*VM =A
*DRAW POINTS (Y, 50:350!2), LINETYPE dashed
*VIEW
```



We have generated a "straight-line" segment of the P vs. t curve for $S_0 = 500$, drawn it, added some "noise", shown the simulated points obtained, fit a straight line to these points, set V_m as the slope of this line, and drawn the straight-line fit.

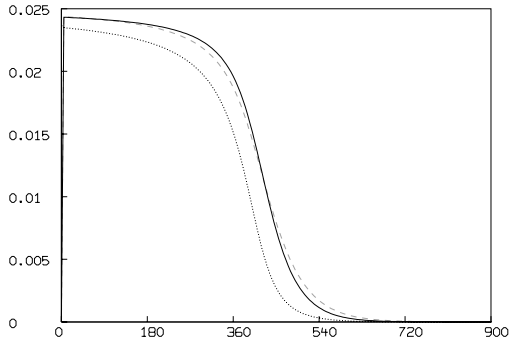
Now, we proceed in the same manner to do another "experiment" to help compute K_m. Type:

```
*DELETE W
*S0 = 10
*Z = INTEGRATE(P DIFF T, C DIFF T,100:300:10) COL 1:2
```

```
*Z COL 2 = (Z COL 2)+(NORMRAN ON 0^^NROWS(Z))/4
*FIT(A,B),Y TO Z, CONSTRAINTS QS
*KM = VM*S0/A-S0\
*TYPE VM,KM
```

Now, V_m and K_m are computed. Let us look at the result. Type:

```
*DRAW Q COL (1,3)
*DRAW (Q COL 1)&'(MM ON SM) LINETYPE dotted color red
*DRAW Q1 COL (1,3) LINETYPE DASHED color green
*VIEW
```



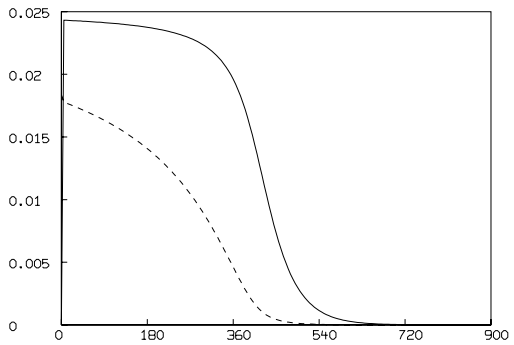
Note the dP/dt curve predicted from the kinetic differential equation model is much better than the consistent underestimate predicted by the Michaelis-Menten model.

There is another approach to estimating the Michaelis-Menten constants, V_m and K_m, based on the intersections of various linear plots. This scheme is due to R. Eisenthal and A. Cornish-Bowden (Biochemistry Journal, Vol. 139, pp. 715:730). It is robust and, at the cost of more experiments, allows a confidence region for V_m and K_m to be obtained, without the usual restrictive assumptions. Unfortunately, it often produces poor extimates of V_m and K_m.

Given observations (S_{0i}, H_i) of substrate concentrations and corresponding product-formation velocities (obtained by linear-regression), we can construct lines defined by $V_m/H_i + K_m/S_{0i} = 1$, which may be plotted in K_m, V_m space. The line $\{\,(K_m, V_m) \mid V_m/H_i + K_m/S_{0i} = 1\,\}$ is the locus of all (K_m, V_m) pairs which could produce the observation (S_{0i}, H_i). Each of the (K_m, V_m) points obtained by the intersections of all pairs of these lines is an estimate of the "true" K_m, V_m values. The arithmetic median of the K_m-estimates is the Eisenthal-Cornish-Bowden estimate of K_m, and the arithmetic median of the V_m-estimates is the Eisenthal-Cornish-Bowden estimate of V_m.

We shall simulate ten experiments for $S_0 = 50 : 500 : 50$ and compute the Michaelis-Menten velocity curve based on K_m and V_m as estimated by the Eisenthal-Cornish-Bowden procedure.

```
*FUNCTION VMF(I,J) = (SV[I]-SV[J])/(SV[I]/VV[I]-SV[J]/VV[J])
*FUNCTION KMF(I,J) = (VV[J]-VV[I])/(VV[I]/SV[I]-VV[J]/SV[J])
*FOR I = 1:10 DO \
{S0 = 50*I;
  Z = INTEGRATE(P DIFF T, C DIFF T, 100:300:10) COL 1:2;
  Z COL 2  = (Z COL 2) + (NORMRAN ON 0^^NROWS(Z))/4;
  LSQRPT = 8;
  FIT(A,B),Y to Z, CONSTRAINTS QS;
  SV[I] = S0; VV[I] = A;
};
*D = 1:9^^'9
*D = COMPRESS((LIST(D)&'LIST(D'))*'LIST(D'<=D))
*D  COL 1 = (D COL 1) +1
*VM = MEDIAN(VMF ON D)
*KM =MEDIAN(KMF ON D)
*TYPE VM,KM
*DELETE W
*DRAW Q COL (1,3)
*DRAW (Q COL 1)&'(MM ON SM),LINETYPE DASHED
*VIEW
```



Overall the best approach to enzyme kinetics is to try to measure enough points on the kinetic curves of several species, so that direct curve-fitting using the appropriate differential equation model can permit the association and dissociation constants to be found. The Michealis-Menten equation is used only due to the difficulty of obtaining data other than $P(t)$ for $t_0 < t < t_2$. Even then, concurrent use of the kinetic model is useful. An excellent source for mathematical models in enzyme kinetics is: Enzyme Kinetics by Kent Plowman, published by McGraw-Hill. Another is Enzyme Kinetics by Irwen Segal, published by Wiley.