

# Chemical Kinetics: Simple Binding: $F + G \rightleftharpoons B$

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## 0.1 Simple Binding

Consider the reaction  $F + G \xrightleftharpoons[k_2]{k_1} B$ .

The study of this reaction is common in chemistry and biochemistry. For example,  $F$  could be a hormone or drug and  $G$  the associated receptor sites. The symbol  $B$  represents the bound complex.

Let us also allow  $F(t)$ ,  $G(t)$ , and  $B(t)$  to be functions which specify the concentrations of  $F$ ,  $G$ , and  $B$  respectively at time  $t$ . Then we have the differential equation model:

$$\begin{aligned}\frac{dB}{dt}(t) &= k_1 F(t)G(t) - k_2 B(t), & B(0) &= B_0, \\ F(t) &= F_0 - (B(t) - B_0), \\ G(t) &= G_0 - (B(t) - B_0),\end{aligned}$$

where  $F_0$ ,  $G_0$ , and  $B_0$  are the initial concentrations of  $F$ ,  $G$ , and  $B$  respectively, and the molar association and dissociation rate constants  $k_1$  and  $k_2$  appear as the proportionality constants for the terms which occur in  $dB/dt$ .

Note that  $k_1 = (\frac{dB}{dt}(0) + k_2 B_0)/(F_0 G_0)$ , so when  $B_0 = 0$  and  $F_0$  and  $G_0$  are known,  $k_1$  may be estimated from the initial velocity  $dB/dt(0)$ , which can, in turn, be estimated from a few points with  $t$  near 0.

The solution to our differential equation is:

$$B(t) = (S(B_0 - R) - R(B_0 - S)e^{d \cdot k_1 \cdot t}) / (B_0 - R - (B_0 - S)e^{d \cdot k_1 \cdot t}),$$

where

$$\begin{aligned} S &= A + d/2 \\ R &= A - d/2 \\ d &= 2(A^2 - (F_0 + B_0)(G_0 + B_0))^{1/2} \\ A &= (F_0 + G_0 + 2B_0 + k_2/k_1)/2 \end{aligned}$$

An appropriate definition of this function in MLAB involves using auxiliary functions as follows:

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fct B(T) = H1((F0+G0+2*B0+K2/K1)/2,T)
fct H1(A,T) = H2(A,SQRT(4*(A^2-(F0+B0)*(G0+B0))),T)
fct H2(A,D,T) = H3(A+D/2,A-D/2,EXP(D*K1*T))
fct H3(S,R,E) = ((B0-R)*S-(B0-S)*R*E)/(B0-R-(B0-S)*E)
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It is useful to study this example carefully. Many occasions will arise where functions will need to be defined with the help of auxiliary functions in a similar manner.

If we have time-course data consisting of time-values *vs.* concentration-values of one or more of the species  $B$ ,  $F$ , or  $G$ , we can use a curve-fitting program like MLAB that handles ODE models, (or in this case, since we have an algebraic model, a program like MLAB that handles non-linear models,) to estimate the values of  $k_1$ ,  $k_2$ , and even  $F_0$  and  $G_0$  and  $B_0$ , (although usually  $B_0$  is 0.) It is actually easier and more transparent to use the ODE model than it is to use the algebraic model.

Note we must have data at early times before equilibrium is approached; data at late times will allow us to estimate the equilibrium constant  $k_1/k_2$ , but  $k_1$  and  $k_2$  will not be separably estimable with only late time data.

Moreover, if we try to estimate all the parameters  $F_0$ ,  $G_0$ ,  $k_1$ , and  $k_2$ , we are likely to get a good pictorial fit, but also estimates that are completely non-unique, where varying any parameter can be compensated by varying the others, so that our estimates are essentially worthless. If we have previously determined  $k_1/k_2$  by fitting an equilibrium model, this will help in reducing the “degrees of freedom”.

Note however, when  $k_1$  and  $k_2$  are known, (or  $k_1/k_2$  in the equilibrium case,) we can use kinetic or equilibrium data measuring any of  $F$ ,  $G$ , or  $B$  to “assay” the initial amounts of  $F$  or  $G$  or both by estimating  $F_0$  and/or  $G_0$  via curve-fitting, (again assuming  $B_0$  is known.) Examples of fitting our kinetic model to data will be given below.

## 0.2 Derivation of the Kinetic Model

Suppose we have a volume with  $\#F$  free  $F$ -molecules and  $\#G$  free  $G$ -molecules and also  $\#B$   $B$ -molecules and  $S$  solvent molecules;  $\#F$  is a function of time,  $\#F(t)$ , and similarly,  $\#G$  is a function of time,  $\#G(t)$ , and  $\#B$  is a function of time,  $\#B(t)$ . But  $S$  is assumed to be constant.

Let  $N(t)$  be the total number of molecules present at time  $t$ ;  $N(t) = \#F(t) + \#G(t) + \#B(t) + S$ . As  $F + G \rightleftharpoons B$ ,  $N(t)$  varies. Note the minimum possible value of  $N(t)$  is  $\#F(0) + \#G(0) + \#B(0) - \min(\#F(0), \#G(0)) + S$  and the maximum possible value of  $N(t)$  is  $\#F(0) + \#G(0) + 2\#B(0) + S$ .

Now for a random “collision” of molecules occurring at or sufficiently near time  $t$ , the probability that this is a collision of an  $F$ -molecule and a  $G$ -molecule is  $\frac{\#F(t)}{N(t)} \cdot \frac{\#G(t)}{N(t)} + \frac{\#G(t)}{N(t)} \cdot \frac{\#F(t)}{N(t)}$ , since we first pick one molecule and then the second to form our collision, and the first is either an  $F$ -molecule and the second is a  $G$ -molecule, or vice-versa. (This is assuming the number of each type of molecule is large enough so that a “sampling with replacement” model is adequate. The “true” probability is  $\binom{\#F}{1} \binom{\#G}{1} / \binom{N}{2} = \frac{2 \cdot \#F \cdot \#G}{N(N-1)}$ . Note  $N$  is a function of  $t$  here and below.)

We take a time-interval  $I_t$  containing  $t$  such that  $I_t$  is short enough that  $N(t)$  does not appreciably change during  $I_t$ .

Now, looking at our stochastic collision process, assume that during the small interval of time  $I_t$  containing  $t$ , the expected number of “collisions” of molecules per second in our volume is proportional to  $N(t)^2 \cdot L_t$ , *i.e.*,  $\alpha N(t)^2 \cdot L_t$ , where  $L_t$  is the length, *i.e.*, the duration, of the time-interval  $I_t$ .

This assumption is acceptable when we also accept that the number of collisions between molecule  $i$  and molecule  $j$  during the time-interval  $I_t$  follows a Poisson distribution with the density parameter  $\lambda L_t$ , *i.e.*,  $P(\text{there are } k \text{ } (i, j)\text{-collisions during } I_t) \approx e^{-\lambda L_t} (\lambda L_t)^k / k!$ . Then the expected number of collisions between molecule  $i$  and molecule  $j$  during the time-interval  $I_t$  is approximately  $\lambda L_t$ . Here  $\lambda$  is an unknown parameter specifying the expected number of collisions between any fixed pair of molecules during a time-interval of unit length. Now since we have  $\binom{N}{2}$  pairs of molecules that can be the components of a collision, the total expected number of collisions during the time-interval  $I_t$  is approximately  $\binom{N}{2} \lambda L_t \approx \alpha N^2 L_t$  where  $\alpha = \lambda/2$ .

The Poisson distribution arises as follows. Let an interval  $I$  of length  $L$  be divided into  $n$  subintervals of length  $\frac{L}{n}$ . Suppose the probability of at least one *event* (such as a collision between two specified particles) in *any* length  $\frac{L}{n}$  subinterval of  $I$  is  $p_n$ . Note  $p_n$  is a function of  $L$  as well as  $n$ . We will assume that the occurrence of *events* in *any* subinterval is *independent* of the occurrence of *events* in any other disjoint subinterval. And also we assume that the expected number of events in an interval of length  $\delta L$  is  $\delta$  times the expected number of events in an interval of length  $L$ ; *i.e.*, let  $\bar{X}_L$  denote the expected number of events in an interval of length  $L$ , then  $\bar{X}_{\delta L} = \delta \bar{X}_L$ .

The probability of at least one event occurring in exactly  $k$  of our  $n$  disjoint length  $\frac{L}{n}$  subintervals is given by  $\binom{n}{k} p_n^k (1 - p_n)^{n-k}$ , and the expected number of non-empty subintervals is  $n p_n$ . That is, when we consider the events in our  $n$  length- $L/n$  adjacent intervals, “discretely-coalesced” (by combining the number of events in each length- $L/n$  subinterval) into a sequence of Bernoulli

random variables  $b_1, b_2, \dots, b_n$  where  $b_j$  is 1 if one or more events occur in the  $j$ -th subinterval of length  $\frac{L}{n}$  and  $b_j$  is 0 if no events occur in the  $j$ -th subinterval, then the sum  $b_1 + b_2 + \dots + b_n$  is binomially-distributed, *i.e.*,  $P(b_1 + \dots + b_n = k) = \binom{n}{k} p_n^k (1 - p_n)^{n-k}$ . As  $n$  approaches  $\infty$ ,  $\sum_{1 \leq j \leq n} b_j$  approaches the number of events in the interval  $L$  and  $\binom{n}{k} p_n^k (1 - p_n)^{n-k} \rightarrow e^{-\lambda L} (\lambda L)^k / k!$  where  $\lambda L = \lim_{n \rightarrow \infty} np_n$ .

(This implies the probability of two events occurring at exactly the same time is negligible, which is true if time is infinitely-divisible, *i.e.*, correctly modeled by the positive real halfline.)

Now when  $\frac{L}{n}$  is small, we may suppose the probability of one or more events in a length  $\frac{L}{n}$  subinterval is small and such that  $p_n \rightarrow 0$  as  $n \rightarrow \infty$ , so that  $\frac{L}{n} \rightarrow 0$ . Also when  $p_n$  is small, we may suppose that  $p_n^2$  is negligibly small. Now, considering a length  $\frac{L}{n}$  subinterval as two length  $\frac{L}{2n}$  subintervals, we see that  $p_n = p_{2n} + p_{2n} - p_{2n}^2$ ; this is the probability that one or more events occur in the first length  $\frac{L}{2n}$  subinterval *or* in the second length  $\frac{L}{2n}$  subinterval, ( $p_{2n}^2$  is subtracted because it is counted twice in  $p_{2n} + p_{2n}$ ). Thus  $p_n < 2p_{2n}$  and  $np_n < 2np_{2n}$ , and in general,  $np_n$  increases monotonically at a diminishing rate as  $n \rightarrow \infty$ . (Note  $np_n$  is the total expected number of events in our interval of length  $L$ , assuming  $p_n^2$  is negligible.) And since  $p_n \rightarrow 0$  as  $n \rightarrow \infty$ , *i.e.*, as  $\frac{L}{n} \rightarrow 0$ ,  $np_n$  increases monotonically to some limit  $\lambda L$  as  $n \rightarrow \infty$  and  $\frac{L}{n} \rightarrow 0$ . (If  $np_n$  increased to  $\infty$  then there would be an infinite number of events expected in the interval  $I$ , which we dismiss as a possibility.) Equivalently  $np_n \rightarrow \lambda$  as  $n \rightarrow \infty$ .

Moreover, since we assume  $p_n$  is the probability of one or more events in *any* interval of length  $\frac{L}{n}$ , where  $L > 0$  is arbitrary, as noted before,  $p_n$  depends on  $L$  as well as  $n$ , and  $p_n$  must increase as  $L$  increases, approaching 1 as  $L \rightarrow \infty$ , and decrease as  $L$  decreases, approaching 0 as  $L \rightarrow 0$ , assuming  $p_n > 0$  for some finite value of  $L$ . For  $L = 1$ , when  $n \rightarrow \infty$ ,  $\lim_{n \rightarrow \infty} np_n = \lambda$  for some non-negative constant  $\lambda$ , and then in general,  $\lim_{n \rightarrow \infty} np_n = \lambda L$  for arbitrary  $L > 0$ , since for  $L = 1$ ,  $np_n \rightarrow \lambda = \bar{X}_1$  as  $n \rightarrow \infty$ , and for arbitrary  $L > 0$ ,  $np_n \rightarrow \bar{X}_L = L\bar{X}_1 = L\lambda$  as  $n \rightarrow \infty$  by assumption.

Recall the probability of at least one event occurring in exactly  $k$  of our  $n$  disjoint length  $\frac{L}{n}$  subintervals is  $\binom{n}{k} p_n^k (1 - p_n)^{n-k}$ , and the expected number of non-empty subintervals is  $np_n$ . As  $n \rightarrow \infty$ ,  $\binom{n}{k} p_n^k (1 - p_n)^{n-k} \rightarrow e^{-\lambda L} (\lambda L)^k / k!$  for  $k \geq 0$  where  $\lambda L = \lim_{n \rightarrow \infty} np_n$ . This is because,

using the binomial theorem to expand  $(1 - p_n)^{n-k}$ , we have:

$$\begin{aligned}
\binom{n}{k} p_n^k (1 - p_n)^{n-k} &= \binom{n}{k} p_n^k \sum_{0 \leq j \leq n-k} \binom{n-k}{k} 1^{n-k-j} (-p_n)^j \\
&= \frac{(n)_k}{k!} p_n^k \sum_{0 \leq i \leq n-k} \frac{(n-k)_i}{i!} (-p_n)^i \\
&= \frac{(n)_k}{k!} \frac{1}{n^k} (np_n)^k \sum_{0 \leq i \leq n-k} \frac{(n-k)_i}{i!} \frac{1}{(n-k)^i} (-(n-k)p_n)^i \\
&\rightarrow \frac{1}{k!} (\lambda L)^k \sum_{0 \leq i \leq \infty} \frac{1}{i!} (-\lambda L)^i \\
&= \frac{(\lambda L)^k}{k!} e^{-\lambda L}
\end{aligned}$$

where  $(a)^m := a(a-1) \cdots (a-m+1)$ , since  $n-k \rightarrow n$ ,  $np_n \rightarrow \lambda L$ ,  $\frac{(n)_k}{n^k} \rightarrow 1$ ,  $\frac{(n-k)_i}{(n-k)^i} \rightarrow 1$ , and  $(-(n-k)p_n)^i \rightarrow (-np_n)^i \rightarrow (-\lambda L)^i$  as  $n \rightarrow \infty$ .

The probability density function  $f(k) := \frac{(\lambda L)^k}{k!} e^{-\lambda L}$  with the parameter  $\lambda$  representing the “density of events”, (*i.e.*,  $\lambda L$  is the expected number of events in an interval of length  $L$ ), is known as the *Poisson density function* with the parameter  $\lambda L$ .

Thus the probability of exactly  $k$  events in an interval of length  $L$  is  $\frac{(\lambda L)^k}{k!} e^{-\lambda L}$ . Note the interval  $I$  can be an interval in space, or as in our situation, an interval of time. The expected number of events during a time-interval of length  $L$  is

$$\begin{aligned}
\sum_{0 \leq k \leq \infty} k \frac{(\lambda L)^k}{k!} e^{-\lambda L} &= \lambda L \sum_{1 \leq k \leq \infty} \frac{(\lambda L)^{k-1}}{(k-1)!} e^{-\lambda L} \\
&= \lambda L \sum_{0 \leq k \leq \infty} \frac{(\lambda L)^k}{k!} e^{-\lambda L} \\
&= \lambda L e^{-\lambda L} \sum_{0 \leq k \leq \infty} \frac{(\lambda L)^k}{k!} \\
&= \lambda L.
\end{aligned}$$

Note we have obtained the distribution of the Poisson random variable  $X_L$  where  $X_L$  is the number of events in the interval  $[0, L]$  where events occur with density  $\lambda$ , *i.e.*,  $E(X_1) = \lambda$ . The uncountable sequence  $\langle X_L \rangle$  defined as  $L$  ranges over the non-negative real numbers  $\mathcal{R}^+$  is a *Poisson Process*. Let  $S_n$  be the waiting time for the  $n$ -th event with  $S_0 = 0$ , and let  $T_j = S_j - S_{j-1}$  for  $j \geq 1$ ;  $T_j$  is the  $j$ -th interarrival time or *increment* and  $S_n = T_1 + T_2 + \cdots + T_n$ . We have  $P(S_n \leq t) = P(X_t > n)$ . Note  $P(T_2 > t \mid T_1 = s) = P(T_2 > t) = P(X_t = 0) = e^{-\lambda t}$ ; this states  $T_2$  is independent of  $T_1$ , and

in general, the random variables  $T_1, T_2, T_3, \dots$  are independent and identically-distributed with the distribution function  $P(T_j \leq t) = 1 - e^{-\lambda t}$ . The Poisson process has *independent increments* and is *memoryless*. No matter what occurred in the past, at any given current time, the waiting time to the next event has the same distribution as  $T_1$ .

Now returning to the reaction  $F + G \rightleftharpoons B$ , of the approximately  $\alpha N(t)^2 \cdot L_t$  collisions during the interval  $I_t$ , the expected number of  $FG$ -collisions is approximately  $\left( \frac{\#F(t)}{N(t)} \cdot \frac{\#G(t)}{N(t)} + \frac{\#G(t)}{N(t)} \cdot \frac{\#F(t)}{N(t)} \right) \cdot \alpha N(t)^2 \cdot L_t = 2\alpha \#F(t)\#G(t) \cdot L_t$   $FG$ -collisions.

The proportionality “constant”  $\alpha$  depends upon the *density* of molecules in our volume. If we were to have  $N$  varying as our volume remained constant then  $\alpha$  would vary accordingly. If, however, our volume also varies to maintain a constant density, as is generally the case for a solution, or more generally, for any gas or liquid maintained at constant pressure and temperature, then  $\alpha$  is constant, (although  $\alpha$  generally depends on temperature.)

Let the fraction  $\beta$  of these  $FG$ -collisions be energetic enough to bind to form a  $B$  molecule.

Also let  $\gamma$  be the proportion of any given number of  $B$  molecules that “decay” into an  $F$  molecule and a  $G$  molecule during our time-interval  $I_t$ . Then the expected number of such decays occurring during our time-interval  $I_t$  is approximately  $\gamma \#B(t) \cdot L_t$ . (Such “decays” are due to both collisions and molecular instability. Although we may question if a molecule can “decay”, including the case of radioactive decay, in the absence of a “stimulus” of some kind.)

Thus the *expected change* in the number of  $B$ -molecules during the time-interval  $I_t$  is

$$\Delta \#B(t) := \beta \alpha 2 \#F(t) \#G(t) \cdot L_t - \gamma \#B(t) \cdot L_t.$$

This is just the expected number of  $B$ -molecules created during the time-interval  $I_t$  minus the number of  $B$ -molecules destroyed during the same time-interval. Note as  $L_t \downarrow 0$ ,  $\Delta \#B(t) \rightarrow 0$ .

Now  $\frac{\Delta \#B(t)}{L_t} = \frac{1}{L_t} (\beta \alpha 2 \#F(t) \#G(t) \cdot L_t - \gamma \#B(t) \cdot L_t)$ , and  $\frac{\Delta \#B(t)}{L_t} \rightarrow \frac{d\#B(t)}{dt}$  as  $L_t \downarrow 0$ , and thus

$$\frac{d\#B(t)}{dt} = \lim_{L_t \downarrow 0} (\beta \alpha 2 \#F(t) \#G(t) - \gamma \#B(t)) = \beta \alpha 2 \#F(t) \#G(t) - \gamma \#B(t).$$

Now we can convert  $\#F$ ,  $\#G$ , and  $\#B$ , to molarity units, (or other equivalent units,) by multiplying by appropriate constants, *e.g.* for  $\#F$ , we divide  $\#F$  by  $6.02214129 \times 10^{23}$  (*Avogadro's number*) to get the amount of  $F$  in units of *moles*. And we may then multiply by the [*molecular-weight of F*] to get the amount of  $F$  in units of grams. (Avogadro's number is the number of molecular-weight- $M$  molecules in  $M$  grams of these molecules.) And to get the *concentration* of  $F$  in molarity units, we divide the number of moles of  $F$  by the *volume in liters* of our solution containing our reacting substances to get the moles of  $F$ -molecules per liter. We do the same form of conversion to express  $\#B$  and  $\#G$  in molarity units.

We may incorporate all these conversion constants together with the unknown proportionality constants  $\alpha$ ,  $\beta$ , and  $\gamma$  into two unknown constants  $k_1$  and  $k_2$ , and then we may write:

$$\begin{aligned}\frac{dB}{dt}(t) &= k_1 F(t)G(t) - k_2 B(t), & B(0) &= B_0, \\ F(t) &= F_0 - (B(t) - B_0), \\ G(t) &= G_0 - (B(t) - B_0),\end{aligned}$$

where  $F(t)$ ,  $G(t)$ , and  $B(t)$  are the molar concentrations of the species  $F$ ,  $G$ , and  $B$  respectively at time  $t$  and  $F_0$ ,  $G_0$ , and  $B_0$  are the initial concentrations of  $F$ ,  $G$ , and  $B$  respectively, and the molar association and dissociation rate constants  $k_1$  and  $k_2$  appear as proportionality constants for the terms which occur in  $dB/dt$ .

Note, in principle, we can determine the product  $\alpha\beta$  if we know  $k_1$ , and we can determine the value of  $\gamma$  if we know  $k_2$ , (along with the volume of solution.) The unit of  $\alpha\beta$  is  $[(\#molecules)(seconds)]^{-1}$  and the unit of  $\gamma$  is  $(seconds)^{-1}$ , so  $\gamma = k_2$ , and we have  $2\alpha\beta = k_1 \cdot (Avogadro's\ number)^{-2} \cdot (solution\ volume\ in\ liters)^{-2}$ .

### 0.3 Units

In the first-order ordinary differential equation  $dB/dt(t) = k_1 F(t)G(t) - k_2 B(t)$ , we have  $F(t)$ ,  $G(t)$  and  $B(t)$  given in molarity units, *i.e.*, *moles/liter* – or *milliliter* or *microliter*, etc. And then  $dB/dt(t)$  has the unit  $(moles/liter)/second$  – or *nanosecond* or *minute* or *hour*, etc. But then  $F(t)G(t)$  has the unit  $moles^2/liter^2$  and thus  $k_1$  must be a constant with the unit  $(liter)/(moles \cdot second)$ , or more generally,  $(liter)/(moles \cdot time \cdot unit)$ , and  $k_2$  must be a constant with the unit  $1/second$ , or more generally,  $(time \cdot unit)^{-1}$ , in order for our differential equation to be dimensionally consistent. Note  $k_1$  and  $k_2$  do not have interconvertible units, thus the values of  $k_1$  and  $k_2$  are not easily comparable as a measure of either the speed or the equilibrium levels of our reaction. However, the bigger  $k_1$  and the smaller  $k_2$ , the more “irreversible” the reaction  $F + G \xrightleftharpoons[k_2]{k_1} B$  is. If  $k_2 = 0$ , the reaction is completely irreversible.

We have the following units in common use.

fraction composition by mass:  $(mass\ of\ solute)/(mass\ of\ solute\ plus\ solvent)$ .

fraction composition by volume:  $(volume\ of\ solute)/(volume\ of\ solution)$ .

Note the volume of the solution is in general not exactly equal to the volume of the solvent plus the volume of the solute, even when both are in the same state of matter, due to various effects.

molarity concentration (moles/liter):  $(moles\ of\ solute)/(liters\ of\ solution)$ .

We compute the moles of a solute by dividing the mass of the solute in grams by the molecular weight of the solute to obtain a number approximately equal to the number of molecules of solute present scaled by the reciprocal of Avogadro’s number; this is the mole amount of the solute.

molality concentration (moles/kg):  $(moles\ of\ solute)/(kg\ of\ solvent)$ .

The molarity for water solvent at 25-degrees C is approximately the same as the molality.

mole fraction composition:  $(\text{moles of solute}_1)/(\sum_i \text{moles of solute}_i)$ .

Here the solvent is taken as another solute.

normality (charge concentration):  $(\text{valence})(\# \text{ions})(\text{moles of solute})/(\text{liters of solution})$ .

Here we focus on either positive ions or negative ions and, in either case, represent their molar concentrations with positive values.

pH ( $-\log_{10}$  charge concentration) [needs work!] Generally only used when the ion being measured is  $H^+$ . Since the equilibrium constant of the reaction  $H_2O \rightleftharpoons H^+ + OH^-$  is approximately  $10^{-14}$ , we have  $[H^+][OH^-] = 10^{-14}[H_2O]$  where here the square brackets denote concentration amounts, and with  $[H_2O] \approx 55.5$  moles/liter, and  $[H^+] = [OH^-]$ , we have  $[H^+] = (10^{-14})^{1/2}$ , so the pH-value is 7 for 1 liter of water. (??) If pH = 14 in 1 liter of solution, then  $[H^+] = 10^{-14}$  which we take to be sufficiently close to 0 for pH = 14 to serve as the upper bound of pH, representing very little  $H^+$  ion present.

#### 0.4 Derivation of the ODE Solution

We will consider the form of our ODE where  $B_0 = 0$  for simplicity. Thus we have

$$\begin{aligned} \frac{dB}{dt}(t) &= k_1 F(t)G(t) - k_2 B(t), & B(0) &= 0, \\ F(t) &= F_0 - B(t), \\ G(t) &= G_0 - B(t), \end{aligned}$$

where  $F_0$  and  $G_0$  are the initial concentrations of  $F$  and  $G$  respectively, and the molar association and dissociation rate constants  $k_1$  and  $k_2$  appear as the proportionality constants for the terms which occur in  $dB/dt$ .

Thus we have the non-linear ODE

$$B'(t) = k_1(F_0 - B(t))(G_0 - B(t)) - k_2 B(t),$$

or equivalently,

$$B' = k_1 F_0 G_0 - [(F_0 + G_0)k_1 + k_2]B + k_1 B^2.$$

Let

$$\begin{aligned} q_0(t) &= k_1 \cdot F_0 \cdot G_0 \\ q_1(t) &= -[(F_0 + G_0) \cdot k_1 + k_2] \\ q_2(t) &= k_1. \end{aligned}$$

Note  $q_0 \neq 0$  and  $q_2 \neq 0$  is assumed, and generally  $q_1 \neq 0$  as well. (Note our ODE is well-defined when  $k_2 = 0$ . And if  $k_1 = 0$ , we have a linear ODE.) We write  $q_0(t)$ ,  $q_1(t)$ ,  $q_2(t)$  this first time to indicate that, in general, the coefficients in our quadratic righthand-side can be functions of  $t$ , although here they are trivial (constant) functions.

Thus we have  $B' = q_0 + q_1 B + q_2 B^2$  with  $B(0) = B_0 = 0$ .



This means  $B'(0) = q_0 + q_1 B_0 + q_2 B_0^2 = q_0$ .

This is a non-linear first-order ordinary differential equation known as a *Riccatti* equation. We can convert this ODE to a linear second-order ODE and this second-order ODE will have constant coefficients when, as here, our Riccatti equation has constant coefficients.

Let  $v = q_2 B$ . Then  $v' = (q_2 B)' = q_2' B + q_2 B' = (q_0 + q_1 B + q_2 B^2)q_2 + q_2' \frac{v}{q_2}$  since  $\frac{v}{q_2} = B$ . (Note here  $q_2$  is constant, so  $q_2' = 0$  and  $v' = q_2 B'$ .)

Thus

$$\begin{aligned} v' &= q_0 q_2 + (q_1 q_2 + q_2') B + q_2^2 B^2 \\ &= q_0 q_2 + (q_1 q_2 + q_2') \frac{v}{q_2} + v^2 \\ &= q_0 q_2 + (q_1 + \frac{q_2'}{q_2}) v + v^2. \end{aligned}$$

Let  $s = q_0 q_2$  and  $r = q_1 + \frac{q_2'}{q_2} = q_1$ . Then  $v' = s + r v + v^2$ .

Now let  $u(t)$  be a function such that  $-\frac{u'}{u} = v$ , i.e.,  $v = (-\log(u))'$ .

Then  $v' = -(\frac{u'}{u})' = -(\frac{u''}{u}) + (\frac{u'}{u^2})u' = -(\frac{u''}{u}) + v^2$ , so  $v' - v^2 = -(\frac{u''}{u})$ .

And thus  $-\frac{u''}{u} = s + r v = s + r(-\frac{u'}{u})$ , so  $-u'' = s u + r(-u')$ , or  $u'' = -s u + r u'$ , or

$$u'' - r u' + s u = 0.$$

And we have  $B = \frac{v}{q_2} = \frac{-u'}{q_2 u}$ , so  $B_0 = \frac{-u'(0)}{q_2(0)u(0)}$ , and  $B'(0) = q_0(0) + q_1(0)B_0 + q_2(0)B_0^2$ , and with  $B_0 = 0$ , we have  $B'(0) =: B'_0 = q_0$ . And also  $-u'(0) = 0$ , i.e.,  $u'(0) = 0$ .

And  $u'' = r u' - s u$ , so  $u''(0) = r u'(0) - s u(0)$ , or  $u''(0) = -s u(0)$  where  $s = q_0 q_2$ . Let  $u(0) = \delta \neq 0$ . Then  $u''(0) = -q_0 q_2 \delta$  and  $u(0) = \frac{q_0}{B'_0} \delta$  since  $\frac{q_0}{B'_0} = 1$ .

So we have  $u(0) = \delta = -u''(0)/(q_0 q_2)$  and  $u'(0) = 0$  as the initial conditions needed for our second-order linear ODE  $u'' - r u' + s u = 0$ .

Now, let us consider how, in general, we may solve a second-order linear ODE:  $y'' + a_1 y' + a_0 y = f$  with the initial values  $y(0)$  and  $y'(0)$  given.

We may proceed as follows. First note that the general solution is given by  $y = y_c + y_p$  where  $y_c$  is the general solution of the *homogeneous* equation  $y'' + a_1 y' + a_0 y = 0$  containing two constants of

integration to be determined, and  $y_p$  is a *particular* solution of the equation  $y'' + a_1y' + a_0y = f$ , *i.e.*,  $y_p$  satisfies  $y'' + a_1y' + a_0y = f$  together with the initial conditions  $y_p(0) = \alpha$  and  $y_p'(0) = \beta$  with  $\alpha$  and  $\beta$  chosen in any way we wish;  $y_c$  is generally called the *complementary solution* of the differential equation  $y'' + a_1y' + a_0y = f$ .

This is because the linear operator  $[D^2 + a_1D + a_0]$  applied to  $y_c$  is 0, and  $[D^2 + a_1D + a_0]$  applied to  $y_p$  is  $f$ , so  $[D^2 + a_1D + a_0]$  applied to  $y_c + y_p$  is  $f$  and also  $y(0) = y_c(0) + y_p(0)$  and  $y'(0) = y_c'(0) + y_p'(0)$ , and these two equations can be used to determine the two unknown constants in  $y_c$ . Thus  $y(t) = y_c(t) + y_p(t)$  is the solution of our ODE. (This follows from the uniqueness of solutions of well-formed initial-condition problems.)

Now  $[D^2 + a_1D + a_0] = [D - \lambda_1][D - \lambda_2]$  where  $\lambda_1$  and  $\lambda_2$  are the roots of the quadratic equation  $x^2 + a_1x + a_0 = 0$ . Since  $(x - \lambda_1)(x - \lambda_2) = 0$ , we have  $\lambda_1\lambda_2 = a_0$  and  $\lambda_1 + \lambda_2 = -a_1$ .

Our ODE can be written in operator form as  $[D - \lambda_2][D - \lambda_1]y = f$ , and the associated homogeneous ODE is  $[D - \lambda_2][D - \lambda_1]y = 0$ . This latter equation is equivalent to  $[D - \lambda_2](y' - \lambda_1y) = 0$ . Let  $w = y' - \lambda_1y$ . Then  $[D - \lambda_2]w = 0$ , or equivalently,  $w' - \lambda_2w = 0$ . But this first-order linear ODE has the general solution  $w(t) = ce^{\lambda_2 t}$  where  $c$  is a constant determinable by the initial condition value  $w(0) = y'(0) - \lambda_1y(0)$  as  $\boxed{c = y'(0) - \lambda_1y(0)}$ .

Now we have  $w = y' - \lambda_1y = ce^{\lambda_2 t}$ . We can multiply by the “integrating factor”  $e^{-\lambda_1 t}$  to obtain  $y'e^{-\lambda_1 t} - \lambda_1ye^{-\lambda_1 t} = ce^{(\lambda_2 - \lambda_1)t}$ , or  $D(y(t)e^{-\lambda_1 t}) = ce^{(\lambda_2 - \lambda_1)t}$ .

Thus, by the Fundamental Theorem of Calculus:  $\int_0^t \frac{d}{du}g(u)du = g(u)|_{u=0}^{u=t} = g(t) - g(0)$ , we have

$$\int_0^t D_s(y(s)e^{-\lambda_1 s})ds = (y(t)e^{-\lambda_1 t}) - d = \int_0^t ce^{(\lambda_2 - \lambda_1)s}ds$$

where  $d = y(0)e^{-\lambda_1 \cdot 0} = y(0)$ .

Thus  $y(t) = \left( \int_0^t ce^{(\lambda_2 - \lambda_1)s}ds + y(0) \right) / e^{-\lambda_1 t}$ .

Now, for  $\lambda_1 \neq \lambda_2$ ,  $\int_0^t ce^{(\lambda_2 - \lambda_1)u}du = \frac{c}{\lambda_2 - \lambda_1}e^{(\lambda_2 - \lambda_1)t} - \frac{c}{\lambda_2 - \lambda_1}$ , because

$$\frac{d}{dt} \left[ \frac{c}{\lambda_2 - \lambda_1}e^{(\lambda_2 - \lambda_1)t} \right] = \frac{c}{\lambda_2 - \lambda_1}(\lambda_2 - \lambda_1)e^{(\lambda_2 - \lambda_1)t} = ce^{(\lambda_2 - \lambda_1)t},$$

and, the Fundamental theorem of Calculus applies,

*i.e.*,

$$\int_0^t ce^{(\lambda_2 - \lambda_1)u}du = \int_0^t \frac{d}{du} \left[ \frac{c}{\lambda_2 - \lambda_1}e^{(\lambda_2 - \lambda_1)u} \right] du = \frac{c}{\lambda_2 - \lambda_1}e^{(\lambda_2 - \lambda_1)t} - \frac{c}{\lambda_2 - \lambda_1}$$

when  $\lambda_1 \neq \lambda_2$ .

Thus  $y(t)e^{-\lambda_1 t} - y(0) = \frac{c}{\lambda_2 - \lambda_1}e^{(\lambda_2 - \lambda_1)t} - \frac{c}{\lambda_2 - \lambda_1}$ , or

$$y(t) = \frac{c}{\lambda_2 - \lambda_1} \left[ e^{(\lambda_2 - \lambda_1)t} - 1 + \frac{\lambda_2 - \lambda_1}{c}y(0) \right] e^{\lambda_1 t} = \left( \frac{c}{\lambda_2 - \lambda_1} \right) \left[ e^{\lambda_2 t} + \left( \frac{\lambda_2 - \lambda_1}{c}y(0) - 1 \right) e^{\lambda_1 t} \right].$$

And thus  $y(t) = c_1 e^{\lambda_2 t} + c_2 e^{\lambda_1 t}$  where  $c_1$  and  $c_2$  are determinable from the initial values  $y(0)$  and  $y'(0)$ ;  $c_1 = \frac{c}{\lambda_2 - \lambda_1}$  and  $c_2 = y(0) - \frac{c}{\lambda_2 - \lambda_1}$  are constants depending on  $c$  which is, in turn, resolvable from the initial condition values  $y(0)$  and  $y'(0)$  as  $c = y'(0) - \lambda_1 y(0)$ .

If  $\lambda_1 = \lambda_2$  then we have  $D(y(t)e^{-\lambda_1 t}) = ce^{(\lambda_2 - \lambda_1)t} = c$ , and then  $\int_0^t D(y(s)e^{-\lambda_1 s}) ds = \int_0^t c ds$ , so  $y(s)e^{(-\lambda_1)s} \Big|_{s=0}^{s=t} = \int_0^t c ds = cs \Big|_{s=0}^{s=t} = ct$ , so  $y(t)e^{(-\lambda_1)t} - y(0) = ct$ , or  $y(t) = [ct + y(0)]e^{\lambda_1 t}$  where  $c = y'(0) - \lambda_1 y(0)$ .

We have thus determined our complementary solution to be  $y_c(t) = c_1 e^{\lambda_2 t} + c_2 e^{\lambda_1 t}$  when  $\lambda_1 \neq \lambda_2$ , and  $y_c(t) = [c_3 t + c_4]e^{\lambda_1 t}$  when  $\lambda_1 = \lambda_2$ , with  $c_1$  and  $c_2$ , or  $c_3$  and  $c_4$ , determinable from the initial-condition values  $y(0)$  and  $y'(0)$ .

We must find our particular solution  $y_p$  by “guessing”, although there are systematic ways to make successful guesses when  $f$  is one of many simple functions. In particular, when  $f(t)$  is a constant  $\alpha$ , we can determine a particular solution of the equation  $y'' + a_1 y' + a_0 y = \alpha$  as  $y_p(t) = \frac{\alpha}{a_1} t$  when  $a_0 = 0$  and  $a_1 \neq 0$ , and  $y_p(t) = \frac{\alpha}{a_0}$  when  $a_0 \neq 0$ . (And  $y_p(t) = \frac{s\alpha}{2} t^2$  when  $a_0 = 0$  and  $a_1 = 0$ .)

---

For our particular case:  $u'' - ru' + su = 0$ , we have  $r \neq 0$  and  $s \neq 0$ , and the right-hand-side “forcing function” is equal to 0 so  $u(t) = u_c(t) + u_p(t)$ , and  $u_p(t) = 0$ , so  $u(t) = u_c(t)$ .

We have the differential operator  $D^2 - rD + s$  which corresponds to the quadratic equation  $x^2 - rx + s = 0$  whose roots are  $\lambda_1 = \frac{r - [r^2 - 4s]^{1/2}}{2}$  and  $\lambda_2 = \frac{r + [r^2 - 4s]^{1/2}}{2}$  where  $s = q_0 q_2 = k_1^2 F_0 G_0$  and  $r = q_1 = -[(F_0 + G_0)k_1 + k_2]$ . Note  $\lambda_1 \lambda_2 = s$  and  $\lambda_1 + \lambda_2 = r$ .

Note we require  $r^2 - 4s > 0$ , *i.e.*,  $(F_0^2 - 2F_0 G_0 + G_0^2)k_1^2 + 2(F_0 + G_0)k_1 k_2 + k_2^2 > 4k_1^2 F_0 G_0$  which always holds when  $F_0 + G_0 > 0$  and  $k_1 > 0$  and  $k_2 > 0$ , or when  $k_1 > 0$  and  $F_0 \neq G_0$ . (If  $F_0 = G_0$  and  $k_2 = 0$  then we have  $\lambda_1 = \lambda_2 = -F_0 k_1$  and the corresponding solution  $u = (ct + u(0))e^{\lambda_1 t}$  holds with  $c = 0$ .)

And when  $r^2 > 4s$ , we have  $\lambda_1 \neq \lambda_2$  and  $u(t) = c_1 e^{\lambda_2 t} + c_2 e^{\lambda_1 t}$  where  $c_1 = \frac{c}{\lambda_2 - \lambda_1}$  and  $c_2 = u(0) - \frac{c}{\lambda_2 - \lambda_1}$  with  $c = u'(0) - \lambda_1 u(0)$ . And we have  $u'(0) = 0$  and  $u(0) = \delta q_0 / B'_0 = -u''(0) / (q_0 q_2)$ , so  $c = -\lambda_1 u(0) = -\lambda_1 \delta \frac{q_0}{B'_0}$ .

And

$$\begin{aligned}
 q_2 B &= -\frac{u'}{u} \\
 &= -\frac{c_1 \lambda_2 e^{\lambda_2 t} + c_2 \lambda_1 e^{\lambda_1 t}}{c_1 e^{\lambda_2 t} + c_2 e^{\lambda_1 t}} \\
 &= -\frac{c_1 \lambda_2 e^{(\lambda_2 - \lambda_1)t} + c_2 \lambda_1}{c_1 e^{(\lambda_2 - \lambda_1)t} + c_2}
 \end{aligned}$$

And  $\lambda_2 - \lambda_1 = [r^2 - 4s]^{1/2}$ ,  $c_1 = \frac{c}{\lambda_2 - \lambda_1}$ ,  $c_2 = \frac{q_0}{B'_0} \delta - \frac{c}{\lambda_2 - \lambda_1}$  and  $c = -\lambda_1 \frac{q_0}{B'_0} \delta$ .

With  $B(0) = B_0 = 0$ , we have  $B'_0 = q_0$ , so  $u(0) = \delta$  and  $c = -\lambda_1 \delta$  and  $c_1 = \frac{-\lambda_1 \delta}{\lambda_2 - \lambda_1}$ , and  $c_2 = \delta - \frac{-\lambda_1 \delta}{\lambda_2 - \lambda_1} = \frac{\lambda_2 \delta}{\lambda_2 - \lambda_1}$ , and then

$$\begin{aligned}
 B &= -\left[ \frac{-\lambda_1 \lambda_2 \delta}{\lambda_2 - \lambda_1} e^{(\lambda_2 - \lambda_1)t} + \frac{\lambda_1 \lambda_2 \delta}{\lambda_2 - \lambda_1} \right] / \left[ \frac{-\lambda_1 \delta}{\lambda_2 - \lambda_1} e^{(\lambda_2 - \lambda_1)t} + \frac{\lambda_2 \delta}{\lambda_2 - \lambda_1} \right] \\
 &= -\left[ \frac{-\lambda_1 \lambda_2}{\lambda_2 - \lambda_1} e^{(\lambda_2 - \lambda_1)t} + \frac{\lambda_1 \lambda_2}{\lambda_2 - \lambda_1} \right] / \left[ \frac{-\lambda_1}{\lambda_2 - \lambda_1} e^{(\lambda_2 - \lambda_1)t} + \frac{\lambda_2}{\lambda_2 - \lambda_1} \right] \\
 &= -\left[ \frac{-\lambda_1 \lambda_2 e^{(\lambda_2 - \lambda_1)t} + \lambda_1 \lambda_2}{-\lambda_1 e^{(\lambda_2 - \lambda_1)t} + \lambda_2} \right].
 \end{aligned}$$

Thus  $B(t) = \frac{\lambda_1 \lambda_2 e^{(\lambda_2 - \lambda_1)t} - \lambda_1 \lambda_2}{-\lambda_1 e^{(\lambda_2 - \lambda_1)t} + \lambda_2}$  where  $\lambda_1 = \frac{r - [r^2 - 4s]^{1/2}}{2}$  and  $\lambda_2 = \frac{r + [r^2 - 4s]^{1/2}}{2}$  with  $s = q_0 q_2 = k_1^2 F_0 G_0$  and  $r = q_1 = -[(F_0 + G_0)k_1 + k_2]$  such that  $\lambda_1 \neq \lambda_2$ . (If  $\lambda_1 = \lambda_2$ , we have  $F_0 = G_0$  and  $k_2 = 0$  and then  $B(t) = F_0 - \frac{F_0}{F_0 k_1 t + 1} = G_0 - \frac{G_0}{G_0 k_1 t + 1}$ .)

## 0.5 Chemical Kinetic Modeling

Suppose we have specific kinetic data, time versus  $B$ -concentration, appearing as the rows of a two-column matrix,  $BM$ . Note time vs.  $G$ -concentration or time vs.  $F$ -concentration can be easily converted to time vs.  $B$ -concentration. In MLAB, if  $GM$  is a 2 column matrix of time vs.  $G$ -concentration for example, we merely type

```
* BM = (GM COL 1)&'(G0-B0-(GM COL 2))
```

and  $BM$  is then the desired time vs.  $B$ -concentration matrix of data points.

We may use the curve-fitting facility of MLAB to compute estimates of  $k_1$  and  $k_2$ , and even  $F_0$ ,  $G_0$ , and/or  $B_0$  if necessary.

---

Given the kinetic data:  $(0, 0), (.2, .072), \dots$  entered below with  $B_0 = 0, F_0 = 1$  and  $G_0 = 1$ , we can estimate  $k_1$  and  $k_2$  as follows.

```
MLAB Mathematical Modeling System, Revision: June 12, 2013
Executing file: /usr/local/lib/mlab/mlab
Copyright: Civilized Software, Inc. (301)962-3711, email: csi@civilized.com
Web-site: WWW.CIVILIZED.COM
```

```
Wed Aug 20 16:00:26 2014
Your current working directory is: /home/knott/
Use FILEDIR to reach any other directory.
'* ' is the command prompt
```

This copy of MLAB belongs to csi choptank

```
d col 1 = 0:4:.2

d col 2 = list(0, .072, .127, .168, .200, \
.170, .187, .205, .176, .197, .165, \
.228, .235, .212, .197, .215, .227, \
.221, .216, .204, .210 )

fct B't(t) = k1*(F0-(B(t) -B0))*(G0-(B(t) -B0)) -k2*B
init B(0) = 0

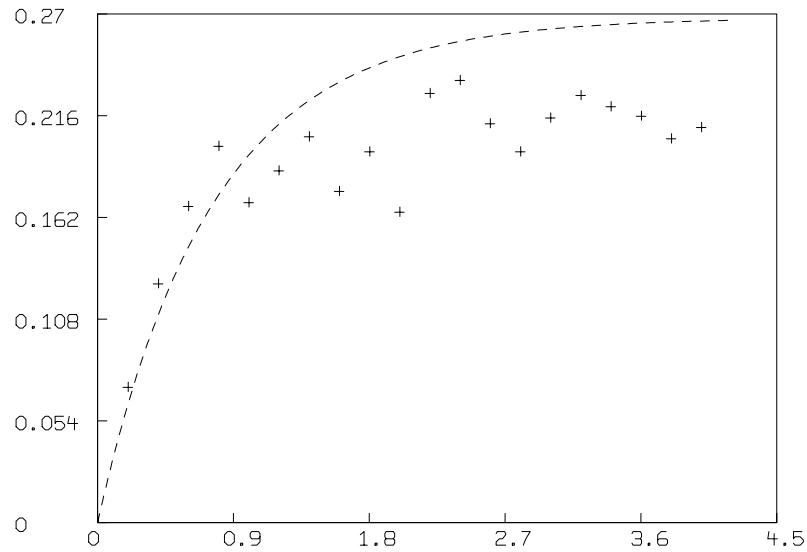
F0 = 1; G0 = 1; B0 = 0

/* Guess k1 = initial slope. */
k1 = .072/.2; k2 = 2*k1

tv = 0:4.2!127

draw d, pointtype "+" linetype none
draw points(B,tv) linetype dashed
view
```

Guess:  $k1 = .36$ ,  $k2 = .72$ , ( $F0 = 1$ ,  $G0 = 1$ , true)



constraints  $q = \{ k1 > 0, k2 > 0, G0 > 0, F0 > 0, B0 \geq 0 \}$

fit( $k1, k2$ ), b to d with weight  $ewt(d)$   
 final parameter values

value	error	dependency	parameter
0.4681184643	0.06528559617	0.9795234833	K1
1.371659988	0.201698861	0.9795234833	K2

4 iterations

CONVERGED

best weighted sum of squares =  $3.156126e+01$

weighted root mean square error =  $1.288844e+00$

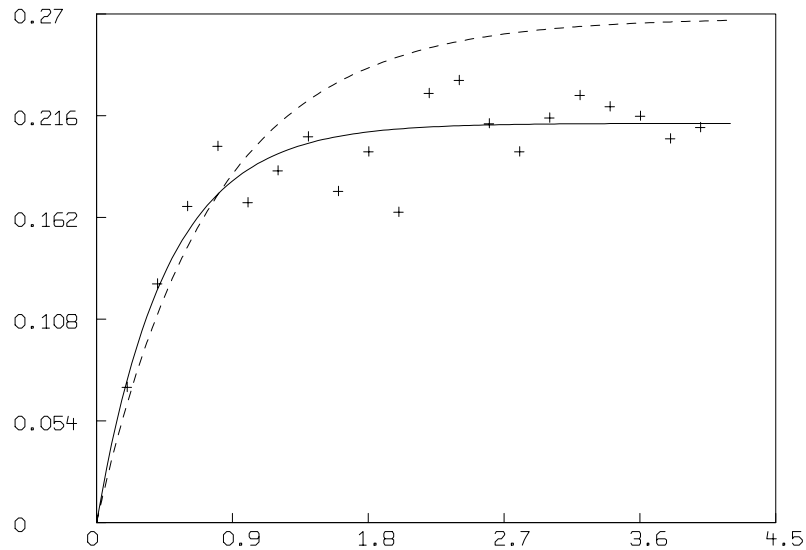
weighted deviation fraction =  $4.233717e-02$

R squared =  $9.098160e-01$

draw points(B, tv) color red

view

Fit:  $k_1 = .468$ ,  $k_2 = 1.37$  ( $F_0 = 1$ ,  $G_0 = 1$ , true)



### 0.5.1 Estimating Initial Condition Values

Now suppose we have the same data, but  $F_0$  is unknown. Then we can estimate  $k_1$ ,  $k_2$ , and  $F_0$  as follows.

```
k1 = .072/.2; k2 = 2*k1; F0 = .5; G0 = 1
```

```
fit(k1,k2,F0), b to d with weight ewt(d), constraints q
final parameter values
```

value	error	dependency	parameter
0.7380400592	9.696694591	0.9999969975	K1
1.176742194	6.870010323	0.999981958	K2
0.6406740748	8.475673511	0.9999984767	F0

```
10 iterations
```

```
CONVERGED
```

```
best weighted sum of squares = 3.148085e+01
```

```
weighted root mean square error = 1.322473e+00
```

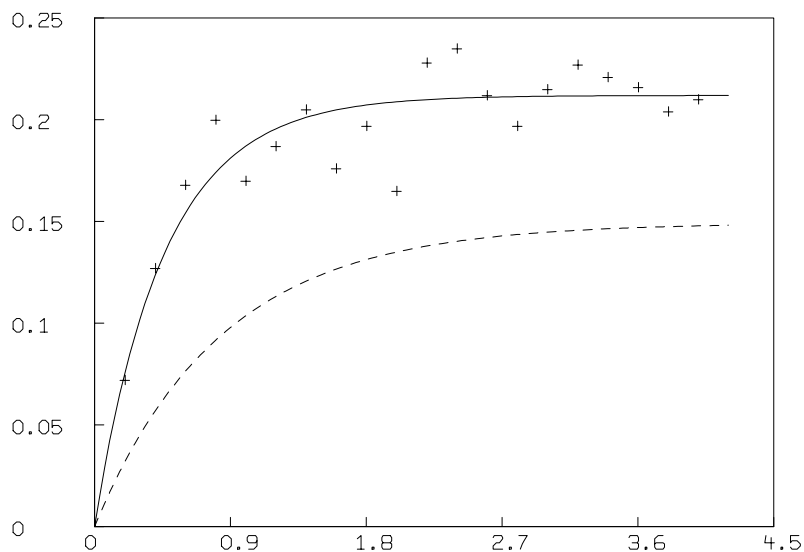
```
weighted deviation fraction = 4.234152e-02
```

```
R squared = 9.100870e-01
```

```
no active constraints
```

```
draw points(B, tv) color yellow linestyle alternate
view
```

Fit:  $k_1 = .738$ ,  $k_2 = 1.177$ ,  $F_0 = .641$  ( $G_0 = 1$ , true)



Note the extremely-high dependency values (greater than .99.) These are matched by very large error values. This means the parameters with such high dependency values are dependent on one-another. Changing one can be compensated by suitably changing the others, *i.e.*, our estimates *are not unique*.

Now let us look at estimating  $k_1$ ,  $k_2$ ,  $F_0$ , and  $G_0$  using the same kinetic data.

```
k1 = .072/.2; k2 = 2*k1; F0 = .5; G0 = .5
```

```
fit(k1,k2,F0,G0), b to d with weight ewt(d), constraints q
final parameter values
```

value	error	dependency	parameter
0.9528428531	10.11946107	0.9999944158	K1
1.106581655	4.324644348	0.9999520682	K2
0.7082117644	406.2857598	0.999999999	F0
0.7082117643	406.2207178	0.999999999	G0

```
6 iterations
```

```
CONVERGED
```

```
best weighted sum of squares = 3.141634e+01
```

```
weighted root mean square error = 1.359419e+00
```

```
weighted deviation fraction = 4.231069e-02
```

```
R squared = 9.102791e-01
```

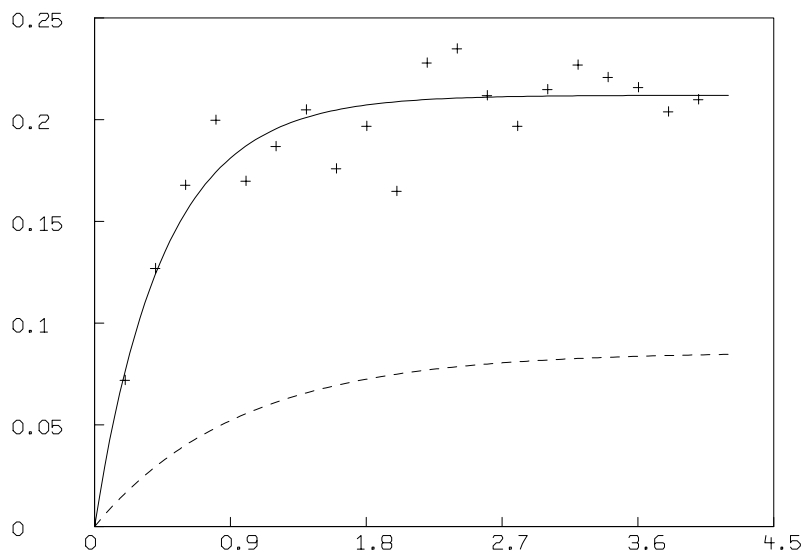
```
no active constraints
```

```
draw points(B, tv) color pink linestyle alternate
```



view

Fit:  $k_1 = .95$ ,  $k_2 = 1.11$ ,  $F_0 = .708$ ,  $G_0 = .708$



Again we have very high dependency values. The values we obtain are not only not well-determined; they depend on the initial guesses.

Finally let us assume  $k_1$  and  $k_2$  are known from prior modeling as  $k_1 = .46812$  and  $k_2 = 1.37166$ , and let us look at estimating  $F_0$ , and  $G_0$  using the same kinetic data as above.

$k_1 = .46812$ ;  $k_2 = 1.37166$ ;  $F_0 = .5$ ;  $G_0 = .5$

fit( $F_0, G_0$ ), b to d with weight ewt(d), constraints q

final parameter values

value	error	dependency	parameter
1.577750772	1.090627947	0.9992427369	$F_0$
0.6650012081	0.3499321856	0.9992427369	$G_0$

4 iterations

CONVERGED

best weighted sum of squares =  $3.172941e+01$

weighted root mean square error =  $1.292273e+00$

weighted deviation fraction =  $4.218845e-02$

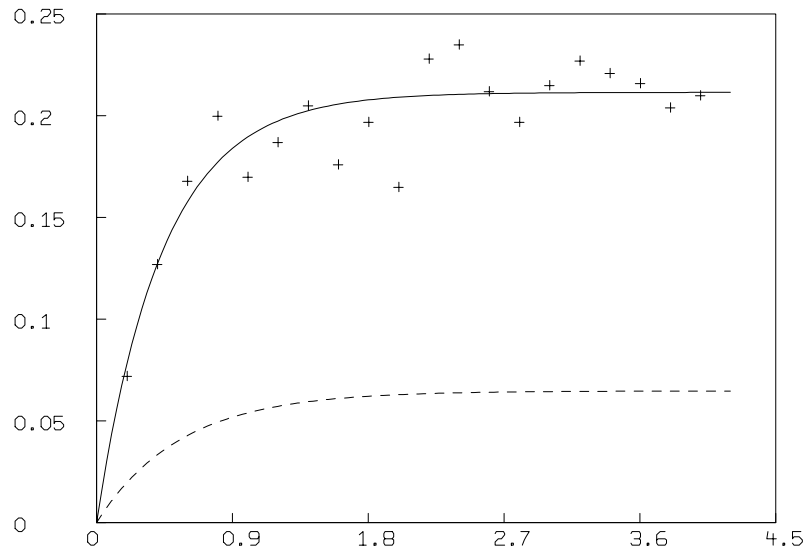
R squared =  $9.102412e-01$

no active constraints

draw points(B, tv) color green

view

Fit:  $F_0 = 1.578$ ,  $G_0 = .665$  ( $k_1 = .468$ ,  $k_2 = 1.372$ )



And yet again we have high dependency values, thus we can estimate  $k_1$  and  $k_2$ , given  $F_0$ ,  $G_0$  and  $B_0$ , reasonably uniquely, but estimating  $F_0$  and  $G_0$  given  $k_1$  and  $k_2$  and  $B_0$  is problematic.

The following example shows that  $G_0$  can be well-estimated when  $k_1$ ,  $k_2$ , and  $F_0$  are accurate.

```
k1 = .5; k2 = 1.5; F0 = 1; G0 = 2
```

```
fit(G0), b to d with weight ewt(d), constraints q
final parameter values
```

value	error	dependency	parameter
1.015055099	0.01600248526	0	G0

```
4 iterations
```

```
CONVERGED
```

```
best weighted sum of squares = 3.199585e+01
```

```
weighted root mean square error = 1.264829e+00
```

```
weighted deviation fraction = 4.222580e-02
```

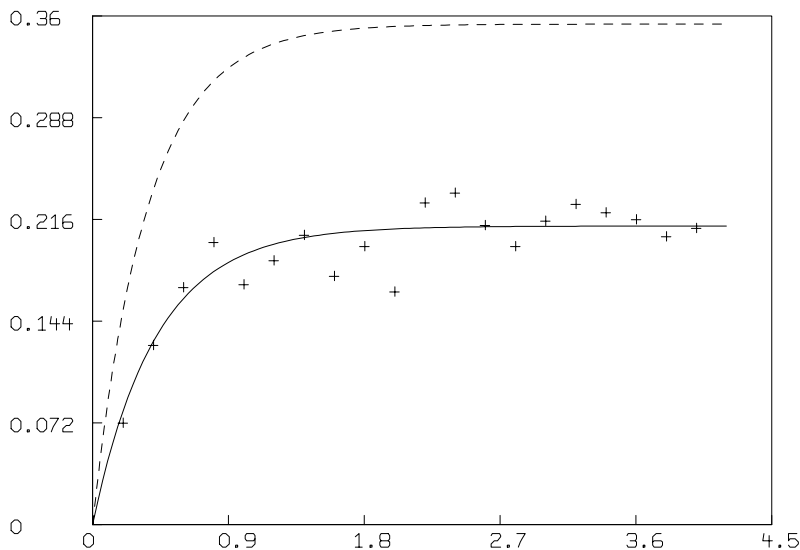
```
R squared = 9.096548e-01
```

```
no active constraints
```

```
draw points(B, tv) color red
```

```
view
```

Fit:  $G_0 = 1.015$  ( $k_1 = .5$ ,  $k_2 = 1.5$ ,  $F_0 = 1$ , true)



## 0.6 Chemical Equilibrium Modeling

The equilibrium constant,  $K$ , of the reaction  $F + G \xrightleftharpoons[k_2]{k_1} B$ , is defined as  $K = k_1/k_2$ . At equilibrium (say at time  $t_e$  where  $t_e$  is suitably large), we have  $dB/dt(t_e) = 0$ , and hence  $k_1F(t_e)G(t_e) - k_2B(t_e) = 0$ . (Of course we may never be *at* equilibrium, so we understand we are simplifying the circumstances.)

Thus,  $\frac{k_2}{k_1} \cdot \frac{B(t_e)}{F(t_e)G(t_e)} = 1$ , so multiplying by  $\frac{k_1}{k_2}$ , we have

$$K = B(t_e)/(F(t_e)G(t_e)) = B(t_e)/((F_0 + B_0 - B(t_e))(G_0 + B_0 - B(t_e))).$$

Thus  $K(F_0 + B_0 - B(t_e))(G_0 + B_0 - B(t_e)) = B(t_e)$  or  $K^{-1}B(t_e) = (F_0 + B_0)(G_0 + B_0) - B(t_e)(G_0 + F_0 + 2B_0) + B(t_e)^2$ .

So  $B(t_e)^2 - ((G_0 + F_0 + 2B_0) + K^{-1})B(t_e) = (F_0 + B_0)(G_0 + B_0) = 0$ , and, using the quadratic formula, we have  $2B(t_e) = (G_0 + F_0 + 2B_0 + K^{-1}) \pm [(G_0 + F_0 + 2B_0 + K^{-1})^2 - 4(F_0 + B_0)(G_0 + B_0)]^{1/2}$ .

Now, to determine the sign + or -, consider the case where  $B_0 > 0$ ,  $F_0 = 0$ , and  $G_0 = 0$ ; in this case,  $B$  will dissociate until equilibrium is reached. Then we have  $B(t_e) \leq B_0$  and  $2B(t_e) = (2B_0 + K^{-1}) \pm [(2B_0 + K^{-1})^2 - 4B_0^2]^{1/2}$ . And, if the plus-sign applies, we have  $2B(t_e) = 2B_0 + K^{-1} + [(4B_0^2 = 4B_0K^{-1} - 4B_0^2)]^{1/2} = 2B_0 + K^{-1} + 2[B_0K^{-1}]^{1/2}$ , so  $B(t_e) = B_0 + \frac{1}{2}K^{-1} + [B_0K^{-1}]^{1/2} > B_0$ . Therefore, the plus-sign cannot be the correct choice of sign, and we have  $2B(t_e) = (G_0 + F_0 + 2B_0 + K^{-1}) - [(G_0 + F_0 + 2B_0 + K^{-1})^2 - 4(F_0 + B_0)(G_0 + B_0)]^{1/2}$ .

Define  $B_e(F_0, G_0, B_0) = B(t_e)$ , the amount of  $B$  at equilibrium or “saturation”. Then we have the following equation, called the *saturation equation*.

$$B_e(F_0, G_0, B_0) = [(F_0 + G_0 + 2B_0 + 1/K) - ((F_0 + G_0 + 2B_0 + 1/K)^2 - 4(F_0 + B_0)(G_0 + B_0))^{1/2}]/2.$$

If we have data points for a so-called saturation curve consisting of pairs of  $F_0$  values with associated  $B_e$  values for fixed values of  $G_0$  and  $B_0$ , then curve-fitting can be used with the above function in order to estimate  $K$ . Indeed  $K$  can be estimated even when we have points  $(F_0, G_0, B_e)$  from the saturation surface in 3-space, where arbitrary values of  $F_0$  and  $G_0$  have been paired, and  $B_0$  is fixed. (Usually,  $B_0 = 0$ .)

Define  $B_e = B(t_e)$ ,  $F_e = F(t_e)$ , and  $G_e = G(t_e)$ . Then from the basic relations:  $K = B_e/(F_e G_e)$ ,  $F_e + B_e = F_0 + B_0$ , and  $G_e + B_e = G_0 + B_0$ , we may write a number of equivalent relationships.

Michaelis-Menten Equation(1) ( $B_e$  vs.  $F_e$ ):

$$B_e = K(G_0 + B_0)F_e/(1 + KF_e)$$

Michaelis-Menten Equation(2) ( $B_e/(B_0 + G_0)$  vs.  $F_e$ ):

$$B_e/(B_0 + G_0) = KF_e/(1 + KF_e)$$

Lineweaver-Burk Equation ( $1/B_e$  vs.  $1/F_e$ ):

$$1/B_e = (1/(K(G_0 + B_0)))(1/F_e) + 1/(G_0 + B_0)$$

Eadie-Wilkinson-Dixon Equation ( $F_e/B_e$  vs.  $F_e$ ):

$$F_e/B_e = F_e/(G_0 + B_0) + 1/(K(G_0 + B_0))$$

Scatchard Equation ( $B_e/F_e$  vs.  $B_e$ ):

$$B_e/F_e = -KB_e + K(G_0 + B_0)$$

Hill Equation ( $\log[(B_e/(G_0 + B_0))/(1 - B_e/(G_0 + B_0))]$  vs.  $\log F_e$ ):

$$\log((B_e/(G_0 + B_0))/(1 - B_e/(G_0 + B_0))) = \log F_e + \log K$$

(Also see the “direct linear plot” of Cornish-Bowden in *Biochem. J.* Vol 137, p. 143, 1974)

Some of these relationships are inspired by analogous relations for enzyme reactions where they arise in different forms. Most are linear relations in  $K$  or  $1/K$  for simple binding, and this accounts for their popularity—they are easy to use as models with linear regression methods in order to estimate  $K$ . For the non-linear Michaelis-Menten forms, constraints are often necessary. *In spite of their traditional use, however, the errors introduced when transforming data to the appropriate form may limit the accuracy obtainable when using any of these models; and in general biased estimates of  $K$  will result.* See Rodbard, D., “Mathematics of Hormone-Receptor Interaction”, in *Receptors for Reproductive Hormones*, Plenum Pub. Corp., NY, 1973.

The saturation equation above may thus be preferred, although usually there is little difference. At any rate, the values of  $K$  obtained using various models should be checked by computing theoretical predicted values for  $B_e$  vs.  $F_0$  and comparing them to the observed values. The major difficulty for the various linear forms is that both the independent and the dependent-variable data values have non-normally-distributed error. As a result, linear Euclidean curve-fitting (with appropriate weights) should be employed with these models. The results should be checked in the saturation equation. That value of  $K$  which yields the lowest sum-of-squares in the saturation model should be used.

### 0.6.1 Curve-Fitting Equilibrium Models

Here is an example comparing the saturation equation model with the Michaelis-Menten (1) model and the Scatchard model.

```
FCT B(F0)=((F0+G0+2*B0+1/K)-SQRT((F0+G0+2*B0+1/K)^2-4*(F0+B0)*(G0+B0)))/2
FCT BE(FE) = (G0+B0)*FE/(1/K+FE)
FCT BS(BE) = -K*BE+K*(G0+B0)

B0 = 0; G0 = 1;

/* M = F0 values, BE values */
M = read(data,100,2)

/* generate M1 = corresponding data (FE,BE) for the Michaelis-Menten model */
M1 = (M COL 1) - (M COL 2) + B0
M1 COL 2 = M COL 2

/* generate M2 = corresponding data (BE/FE,BE) for the Scatchard model */
M2 = (M COL 2)
M2 COL 2 = (M1 COL 2) / (M1 COL 1)

K = 2
FIT(K), B TO M
final parameter values
      value          error          dependency    parameter
      1.996602727    0.0420400256          0          K
1 iterations
CONVERGED
best weighted sum of squares = 1.446128e-02
weighted root mean square error = 1.925622e-02
weighted deviation fraction = 1.849765e-02
R squared = 9.691570e-01
KS = K
```

FIT(K), BE TO M1

final parameter values

value	error	dependency	parameter
2.0043749954	0.0397621839	0	K

1 iterations

CONVERGED

best weighted sum of squares = 1.769429e-02

weighted root mean square error = 2.130023e-02

weighted deviation fraction = 2.026365e-02

R squared = 9.622617e-01

KM = K

FIT(K), BS TO M2

final parameter values

value	error	dependency	parameter
2.0008431423	0.0360417006	0	K

1 iterations

CONVERGED

best weighted sum of squares = 1.242737e-01

weighted root mean square error = 5.644913e-02

weighted deviation fraction = 8.426590e-02

R squared = 9.358569e-01

KC = K

/\* draw MM model + data with the above K \*/

K = KS

TOP TITLE "Saturation Model"

DRAW M, LINETYPE NONE, POINTTYPE STAR

DRAW POINTS(B, 1:5!101)

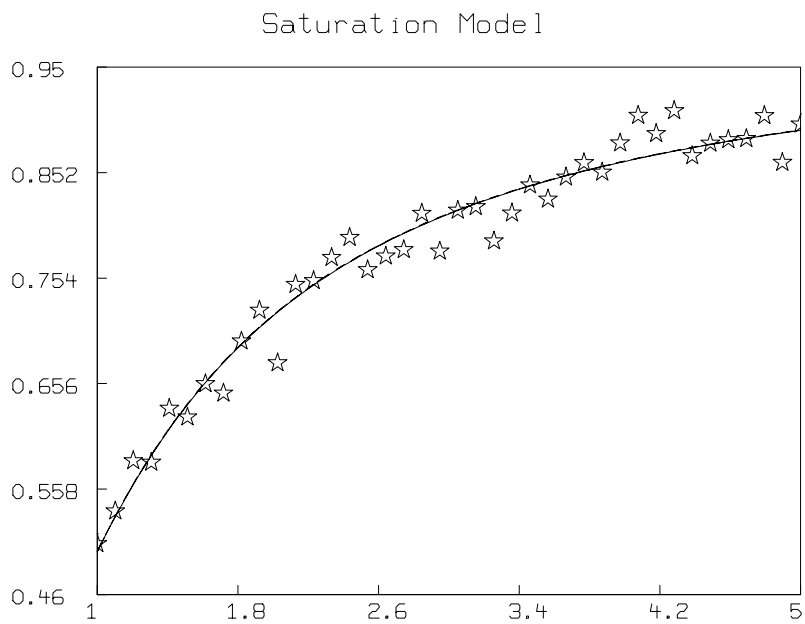
K = KM

DRAW POINTS(B, 1:5!101) LINETYPE DASHED

K = KC

DRAW POINTS(B, 1:5!101) LINETYPE ALTERNATE

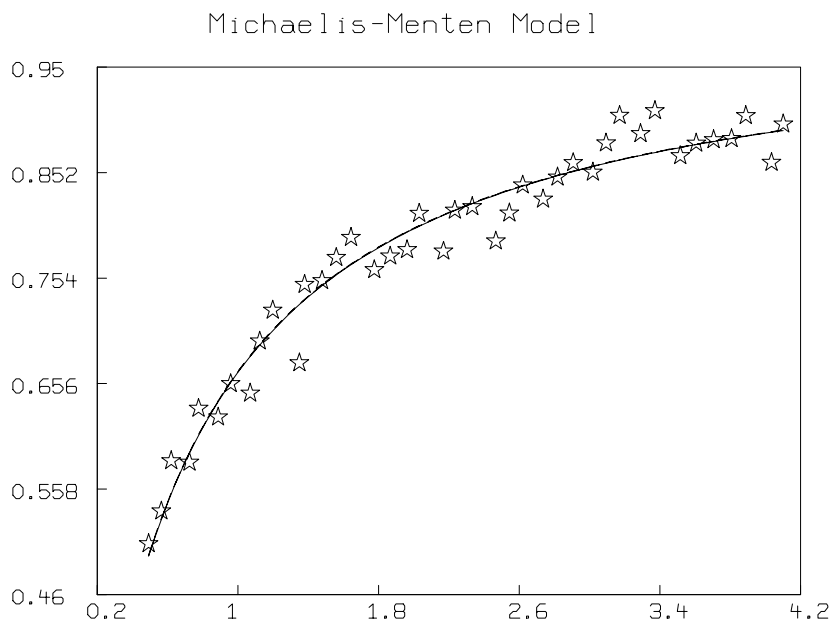
VIEW



```

K = KS
TOP TITLE "Michaelis-Menten Model"
DRAW M1, LINETYPE NONE, POINTTYPE STAR
DRAW POINTS(BE, MINV(M1 col 1):MAXV(M1 col 1)!101)
K = KM
DRAW POINTS(BE, MINV(M1 col 1):MAXV(M1 col 1)!101) LINETYPE DASHED
K = KC
DRAW POINTS(BE, MINV(M1 col 1):MAXV(M1 col 1)!101) LINETYPE ALTERNATE
VIEW

```

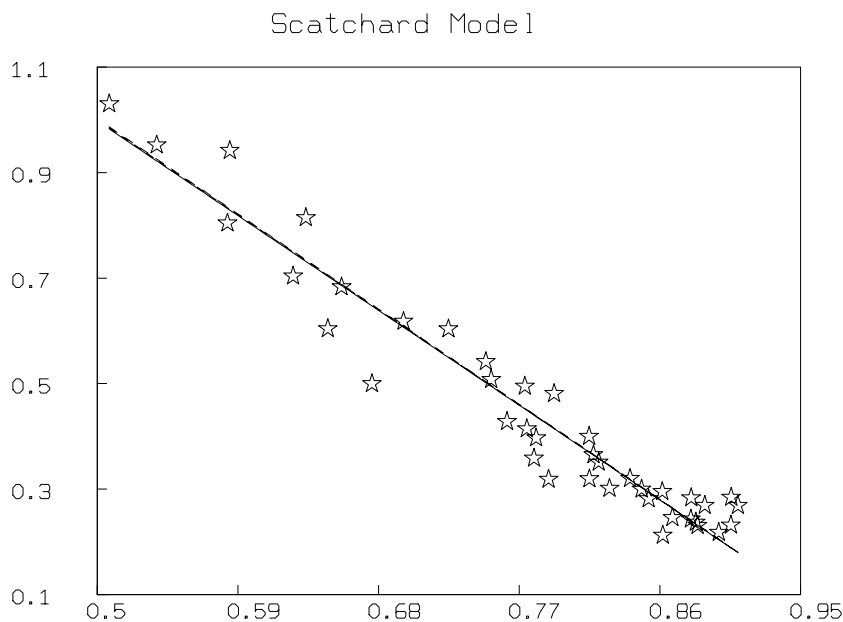


```

/* draw Scatchard model + data with the above K */
K = KS
TOP TITLE "Scatchard Model"
DRAW M2, LINETYPE NONE, POINTTYPE STAR
DRAW POINTS(BS, MINV(M2 col 1):MAXV(M2 col 1)!101)
K = KM
DRAW POINTS(BS, MINV(M2 col 1):MAXV(M2 col 1)!101) LINETYPE DASHED
K = KC
DRAW POINTS(BS, MINV(M2 col 1):MAXV(M2 col 1)!101) LINETYPE ALTERNATE
VIEW

```





Note that for the data studied above, all the models give comparable results. Indeed the estimated  $K$ -values are so close, the curves are almost superimposed.

The second Michaelis-Menten equation is useful when the amount,  $G_0 + B_0$ , is not known. Then measuring a quantity proportional to  $B_e/(G_0 + B_0)$  vs.  $F_e$  is commonly done, and  $K$  can be determined in unknown units. Indeed, by introducing another parameter,  $D$ , to obtain  $B_e/(B_0 + G_0) = DKF_e/(1 + KF_e)$  and computing  $D$  and  $K$  by fitting this model to data points  $(F_e, B_e/(B_0 + G_0))$ , where  $F_e$  and  $B_e$  are measured in moles and  $B_0 + G_0$  is measured in grams, then  $D(B_0 + G_0)$  has the unit moles, and so  $(B_0 + G_0)/(D(B_0 + G_0)) = 1/D$  is the molecular weight of a  $G$  molecule. (This device for computing molecular weight assumes that  $B_0 = 0$  or that an  $F$  molecule is much lighter than a  $G$  molecule.) Fitting the saturation equation to obtain both  $K$  and  $G_0 + B_0$  may be a better approach.

## 0.7 Cooperative Binding

Often the binding of  $F$  and  $G$  is complicated by cooperative effects. Namely,  $k_1$  and/or  $k_2$  appear to be dependent upon the relative amount of  $B$ . This can be due to allosteric shape changes in the molecules or sites  $G$  which occur during binding. Various other explanations, including multiple classes of sites, are also possible. If  $k_1/k_2$  increases as  $B$  increases, we have positive cooperativity; if  $k_1/k_2$  decreases as  $B$  increases we have negative cooperativity.

Suppose, then, that  $k_1$  and  $k_2$  are functions of  $B$ . Thus,

$$dB/dt(t) = k_1(B(t)) \cdot F(t) \cdot G(t) - k_2(B(t)) \cdot B(t),$$

with  $F(t) = F_0 - (B(t) - B_0)$ ,  $G(t) = G_0 - (B(t) - B_0)$ , and  $B(0) = B_0$ , as before.

Now suppose that all cooperative effects are due to changes in  $k_2$ , in particular, suppose

$$k_1(B) = k_1^0, \quad \text{and} \quad k_2(B) = k_2^0(1 + p \cdot B/(G_0 + B_0)).$$

This is the same as saying  $k_2(B) - k_2(0) = pB/(G_0 + B_0)$ . Thus we assume that the change in  $k_2$  from the ‘‘ground state’’  $k_2(0) = k_2^0$ , is proportional to the fraction of occupied sites,  $B/(G_0 + B_0)$ , with the proportionality-constant  $p$ .

Note then, that  $dk_2/dt(B(t)) = (p/(G_0 + B_0))dB/dt(t)$ , so  $dk_2/dB(B) = p/(G_0 + B_0)$ .

There are of course many other functional relationships which could be postulated. For example, we could assume that  $dk_2/dt(B(t)) = A(dB/dt)h$ , or we could assume  $k_1$  and  $k_2$  vary together in certain ways. Indeed, changes in  $k_1$  will give cooperative effects unobtainable by changes in  $k_2$  alone.  $k_1$  and  $k_2$  need not change monotonically; we may have variation which results in intervals of positive cooperativity and other intervals of negative cooperativity.

Since cooperativity, without qualification as to its cause, is merely a mathematical description, and not a structural description, the choice of how  $k_1(B)$  and  $k_2(B)$  are defined is dependent upon the actual physical situation and the desired uses of the mathematical model. The particular choice here has the same effect as that made by DeMeyts in his analysis of cooperativity (DeMeyts, P., Woebroeck, M., ‘‘The structural basis of insulin-receptor binding and cooperative interactions’’, in Membrane Proteins (ed. P.Nicholls et al.) FEBS 11th Meeting, Vol. 45 Symposium A4, Pergamon Press, pp. 319–323, 1977).

Now let  $t_e$  be the time when equilibrium is approached, and let  $B(t_e) = B_e$ ,  $F(t_e) = F_e$ , and  $G(t_e) = G_e$ . Then, at equilibrium, we have the equilibrium constant  $K$  as a function of  $B_e$ ,  $K(B_e) = k_1(B_e)/k_2(B_e) = B_e/(F_e G_e)$ . Thus,  $K(B_e) = k_1^0/(k_2^0(1 + pB_e/(G_0 + B_0)))$ , or,  $K(B_e) = k_0/(1 + pB_e/(G_0 + B_0))$ , where  $k_0 = K(0)$ .

Note for  $-1 < p < 0$ , we have positive cooperativity, for  $p = 0$ , we have no cooperativity ( $K(B_e) = K(0)$ ), and for  $p > 0$ , we have negative cooperativity.

It is convenient to define  $p$  in terms of another parameter,  $a$ , called the  $F$ ,  $G$  interaction factor, so that  $p = (1 - a)/a$ , and hence  $a = 1/(1 + p)$ . Note for  $0 < a < 1$ , we have negative cooperativity, for  $a = 1$ , we have no cooperativity, and for  $a > 1$ , we have positive cooperativity.

Indeed, if  $\Delta G_0$  is the energy needed (or released) (i.e., the change in free energy) for binding the first  $F$  molecule to a  $G$  molecule, and if  $\Delta G_1$  is the energy used (or released) for binding an  $F$  molecule to the last unoccupied  $G$  molecule (whereupon  $B = G_0 + B_0$ ), then we have the classical thermodynamic relations:  $\Delta G_0 = -RT \log K(0)$ , and  $\Delta G_1 = -RT \log K(G_0 + B_0)$ , where  $R$  is the gas constant (about 1.987 calories/degree/mole) and  $T$  is the absolute temperature. Thus,  $K(G_0 + B_0)/K(0) = \exp(-(\Delta G_1 - \Delta G_0)/RT)$ , and, by assumption,  $K(G_0 + B_0)/K(0) = 1/(1 + p) = a$ , so  $a$  has the interpretation:  $a = K(B_0 + G_0)/K(0)$ ; it is the ratio of the equilibrium constant  $K$  with all  $G$ -sites occupied, to  $K$  with no occupied  $G$ -sites.

DeMeyts has observed that the relation  $K(B_e) = k_0/(1 + ((1 - a)/a) \cdot B_e/(G_0 + B_0))$  may be used in the various equilibrium models given before to obtain the corresponding cooperative models.

Thus, we substitute  $K(B_e)$  for  $K$  to obtain models which now involve the parameters  $F_0$ ,  $G_0$ ,  $B_0$ ,  $a$ , and  $k_0$ . Curve-fitting which yields a value for  $a$  obviously different from one, should be followed by fitting with  $a$  fixed to one. If the latter fit is clearly inferior, cooperative binding phenomena may be present.

In particular, for  $a \neq 1$ , the cooperative Michaelis-Menten(2) relation is:

$$B_e/(B_0 + G_0) = (-a(1 + k_0F_e) + [a^2(1 + k_0F_e)^2 + 4a(1 - a)k_0F_e]^{1/2})/(2(1 - a)).$$

The cooperative Scatchard equation is:

$$B_e/F_e = k_0(B_0 + G_0 - B_e)/(1 + (1 - a)B_e/(a(G_0 + B_0))).$$

The cooperative Hill equation is obtainable by substitution from the cooperative Michaelis-Menten (2) equation above, however, it can be plotted in MLAB, without using explicit algebra, as follows, assuming  $B_0$ ,  $G_0$ ,  $k_0$ , and  $a$  are already set, with  $a \neq 1$ .

```
FCT BE(FE) = (B0+G0)*(-A*(1+K0*FE) + \
  SQRT(A*A*(1+K0*FE)^2+4*A*(1-A)*K0*FE))/(2*(1-A))
FUNCTION HILLT(BE) = LOG((BE/(G0+B0))/(1-BE/(G0+B0)))
LFEV = -12:2:.2
M = LFEV &' (HILLT ON BE ON EXP ON LFEV)
DRAW M
```

The Hill-plot matrix  $M$  has rows which are points of the form:

$$\log((B_e/(G_0 + B_0))/(1 - B_e/(G_0 + B_0))) \quad \text{vs.} \quad \log F_e.$$

Normally a Hill-plot, as defined above, is a straight line with slope 1, however, this is not the case for  $a \neq 1$ . DeMeyts has shown that, in general, the slope

$$d(\log((B_e/(G_0 + B_0))/(1 - B_e/(G_0 + B_0))))/d(\log F_e)$$

decreases when  $a$  decreases and increases when  $a$  increases, and attains its minimum value when  $F_e = 1/k_0$ , independently of  $a$ .