



Binding/Saturation Curves

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Purpose

The purpose of this document is to:

1. Provide some background that highlights the areas in which “binding curve” functions are applicable.
2. Provide derivations for the binding/saturation curves that are implemented in **FitAll**.

Background

Binding/Saturation curves are applicable when fitting data that describes::

- The binding of a ligand, molecule or ion to a metal ion.
- The binding of a drug or molecule to DNA.
- The binding or adsorption of a molecule to a surface.
- The binding of one molecule to another that results in either the inhibition or catalysis (acceleration) of a reaction or process.
- The effectiveness of a drug in preventing or delaying the progress of a (fatal) condition.
- The relative reactivity of (intermediate) species that are formed in the course of a chemical reaction.
- The dependence of the reaction rate on acidity (pH or $[H^+]$).

The above are just some examples in which experimental data may be described by binding/saturation curves.

The following sections show the derivations of the Binding/Saturation Curves that are implemented in **FitAll**.

In some areas of study a Dissociation Constant, K_d , rather than a Formation Constant, Q , is used to describe the equilibrium/binding process. The relationship between K_d and Q is: $K_d = 1/Q$.

1. Single Binding/Saturation Curve

The following shows how the fitting function is derived (arrived at) for a simple, single binding system.

The system to be described is the reversible equilibrium equation/reaction:



; that is, the species A and B reversibly combine with a 1:1 stoichiometry to form C.

ASSUME that:

1. The total, initial, concentration of A is A_0 and it is the same for all data points in a given experiment/test.
2. The total concentration of B, B_0 , changes from one data point to the next.
3. Initially, that is when $B_0 = 0$, the concentration of C is zero. That is, no C is present until B has been added to the solution/mixture.

The result of the above is that for any given data point, the concentration of the various species are:

$$[C] = x \quad \dots(1.3)$$

$$[B] = B_0 - x \quad \dots(1.4)$$

$$[A] = A_0 - x \quad \dots(1.5)$$

Define the equilibrium quotient, Q, (aka the formation constant) as:

$$Q = [C] / ([A] * [B]) \quad \dots(1.6)$$

Q can now be expressed in terms of the total concentrations of A, A_0 , and B, B_0 , that have been admixed in the solution to obtain:

$$Q = x / ((A_0 - x) * (B_0 - x)) \quad \dots(1.7)$$

ASSUME that the concentration of B is always very much greater than that of A; that is, $B_0 \gg A_0$. Typically this means that B_0 is at least 10 times A_0 ($B_0 \geq 10 * A_0$).

Note:
If this assumption cannot be made, **FitAll** has two functions (0405 and 0406) in the Chemistry Functions Library that do not make it.

Applying the above assumption to eq 1.7 results in:

$$Q = x / ((A_0 - x) * B_0) \quad \dots(1.8)$$

or

$$x = A_0 * Q * B_0 / (1 + Q * B_0) \quad \dots(1.9)$$

At this point it is necessary to relate the concentrations of species A, B and C to a physical property that can be (easily) measured.

The measured, species specific, response, R_i , can be a measure of any property the follows Beer's Law; that is, a property that is directly proportional to the concentration of that species. It could, for example, be the molar fluorescence, the molar absorptivity, the molar conductivity or the reaction rate constant.

Define the species specific response factors as R_a , R_b and R_c for the species A, B and C, respectively.

This means that the total measured response, R , is given by:

$$R = R_a[A] + R_b[B] + R_c[C] \quad \dots(1.10)$$

ASSUME, as is often the case, that $R_b = 0$.

Applying this assumption means that the total response is given by:

$$R = R_a[A] + R_c[C] \quad \dots(1.11)$$

or

$$R = R_a(A_o - x) + R_c x \quad \dots(1.12)$$

or

$$R = R_a A_o + (R_c - R_a)x \quad \dots(1.13)$$

Substitute the value of x (eq 1.9) into the eq 1.13 to obtain:

$$R = R_a A_o + (R_c - R_a) A_o Q B_o / (1 + Q B_o) \quad \dots(1.14)$$

Let

$$R_o = R_a A_o; \text{ that is, the response when } B_o = 0. \quad \dots(1.15)$$

$$R_{inf} = R_c A_o; \text{ that is, the response when } B_o = \text{infinity}. \quad \dots(1.16)$$

and

$$\Delta R = (R_c - R_a) A_o; \text{ that is, the difference in the response when } B_o \text{ is infinitely large and zero } (R_{inf} - R_o). \quad \dots(1.17)$$

Then

$$R = R_o + \Delta R Q B_o / (1 + Q B_o) \quad \dots(1.18)$$

Additional Notes

1. This is the same as **FitAll** Research Edition's functions 0203 and 0206 with one binding curve, in which

$$\begin{aligned} Y &= R \\ X &= B_o \\ P1 &= R_o \\ P2 &= Q \\ P3 &= \Delta R \end{aligned}$$

2. If it is ASSUMED that $R_a = 0$ then this is the same as **FitAll** Research Edition's functions 0202 and 0205 with one binding curve, in which

$$\begin{aligned} Y &= R \\ X &= B_o \\ P1 &= Q \\ P2 &= \Delta R \end{aligned}$$

3. When 50% of A is bound to B the concentration of B is equal to $1/Q$ or K_d .
4. When $R_a = 0$ and $R_{inf} = R_c A_o = 1.0$ the binding curve is called a "Langmuir Absorption Isotherm". This is often used to describe the absorption of an inhibitor on to a metal or other surface. (See **FitAll's** functions 0003 and 0201).

2. Multiple Uncoupled Binding/Saturation Curves

The following shows how the fitting function is derived (arrived at) for a multiple, uncoupled binding system.

The example system to be described has two reversible equilibrium equations/reactions:



and



; that is, the species A1 and B reversibly combine, with a 1:1 stoichiometry, to form C1 and the species A2 and B reversibly combine, with a 1:1 stoichiometry, to form C2.

The difference between the species A1 and A2 may not initially be obvious or detectable when using standard measurement/analysis techniques.

For example:

1. A1 and A2 may be two different forms of an enzyme (i.e., isozymes) that are not distinguishable when using standard assay methods.
2. A2 may be an impurity in a preparation that is thought to contain only A1.

ASSUME that:

1. The total, initial, concentration of A1 is A1o, A2 is A2o and that both A1o and A2o are the same for all data points in a given experiment/test.
2. The total concentration of B, Bo, changes from one data point to the next.
3. Initially, that is when Bo = 0, the concentration of C1 and C2 are zero. That is, no C1 or C2 is present until B has been added to the solution/mixture.

The result of the above is that for any given data point, the concentration of the various species are:

$$[C1] = x1 \quad \dots(2.5)$$

$$[B] = Bo - x1 - x2 \quad \dots(2.6)$$

$$[A1] = A1o - x1 \quad \dots(2.7)$$

$$[C2] = x2 \quad \dots(2.8)$$

$$[B] = Bo - x1 - x2 \quad \dots(2.9)$$

$$[A2] = A2o - x2 \quad \dots(2.10)$$

Define the equilibrium quotients, Q1 and Q2, (aka the formation constants) as:

$$Q1 = [C1] / ([A1] * [B]) \quad \dots(2.11)$$

and

$$Q2 = [C2] / ([A2] * [B]) \quad \dots(2.12)$$

The Q's can now be expressed in terms of the total concentrations of A1, A1o, A2, A2o, and B, Bo, that have been admixed in the solution to obtain:

$$Q1 = x1 / ((A1o - x1) * (Bo - x1 - x2)) \quad \dots(2.13)$$

$$Q2 = x2 / ((A2o - x2) * (Bo - x1 - x2)) \quad \dots(2.14)$$

ASSUME that the concentration of B is always very much greater than that of A1 and A2; that is, $Bo \gg (A1o + A2o)$. Typically this means that Bo is at least 10 times $A1o$ plus $A2o$; that is, $Bo \geq 10 * (A1o + A2o)$.

Applying the above assumption to eq 2.7 results in:

$$Q1 = x1 / ((A1o - x1) * Bo) \quad \dots(2.15)$$

or

$$x1 = A1o * Q1 * Bo / (1 + Q1 * Bo) \quad \dots(2.16)$$

and

$$Q2 = x2 / ((A2o - x2) * Bo) \quad \dots(2.17)$$

or

$$x2 = A2o * Q2 * Bo / (1 + Q2 * Bo) \quad \dots(2.18)$$

At this point it is necessary to relate the concentrations of species A1, A2, B, C1 and C2 to a physical property that can be (easily) measured.

The measured, species specific, response, Ri , can be a measure of any property the follows Beer's Law; that is, a property that is directly proportional to the concentration of that species. It could, for example, be the molar fluorescence, the molar absorbtivity, the molar conductivity or the reaction rate.

Define the species specific response factors as $R1a$, $R2a$, Rb , $R1c$ and $R2c$, respectively, for the species A1, A2, B, C1 and C2.

This means that the total measured response, R , is given by:

$$R = R1a * [A1] + Rb * [B] + R1c * [C1] + R2a * [A2] + R2c * [C2] \quad \dots(2.19)$$

ASSUME, as is often the case, that $Rb = 0$.

Applying this assumption means that the total response is given by:

$$R = R1a * [A1] + R1c * [C1] + R2a * [A2] + R2c * [C2] \quad \dots(2.20)$$

or

$$R = R1a * (A1o - x1) + R1c * x1 + R2a * (A2o - x2) + R2c * x2 \quad \dots(2.21)$$

Substitute the values of $x1$ (eq 2.16) and $x2$ (eq 2.18) into eq 2.19 to obtain:

$$R = R1a * A1o + R2a * A2o + (R1c - R1a) * A1o * Q1 * Bo / (1 + Q1 * Bo) + (R2c - R2a) * A2o * Q2 * Bo / (1 + Q2 * Bo) \quad \dots(2.22)$$

Let

$$Ro = R1a * A1o + R2a * A2o; \text{ that is the response when } Bo = 0. \quad \dots(2.23)$$

$$Rinf = R1c * A1o + R2c * A2o; \text{ that is the response when } Bo = \text{infinity}. \quad \dots(2.24)$$

and

$$\Delta R1 = (R1c - R1a) * A1o \quad \dots(2.25)$$

$$\Delta R2 = (R2c - R2a) * A2o \quad \dots(2.26)$$

Then

$$R = Ro + \Delta R1 * Q1 * Bo / (1 + Q1 * Bo) + \Delta R2 * Q2 * Bo / (1 + Q2 * Bo) \quad \dots(2.27)$$

Additional Notes

1. This is the same as **FitAll** Research Edition's function 0206 with two binding curves, in which

$$\begin{aligned} Y &= R \\ X &= B_0 \\ P_1 &= R_0 \\ P_2 &= Q_1 \\ P_3 &= \Delta R_1 \\ P_4 &= Q_2 \\ P_5 &= \Delta R_2 \end{aligned}$$

2. If it is ASSUMED that $R_{1a} = 0$ and $R_{2a} = 0$ then this is the same as **FitAll** Research Edition's function 0205 with two binding curves, in which

$$\begin{aligned} Y &= R \\ X &= B_0 \\ P_1 &= Q_1 \\ P_2 &= \Delta R_1 \\ P_3 &= Q_2 \\ P_4 &= \Delta R_2 \end{aligned}$$

3. If there are more than two species/products formed the resulting equation will have a similar form, but have more parameters, P, to resolve.

3. Multiple Coupled Binding/Saturation Curves

The following shows how the fitting function is derived (arrived at) for a multiple, uncoupled binding system.

The system to be described by the two reversible equilibrium equations/reactions:



and



or



and



; that is, the species A and B reversibly combine, with a 1:1 stoichiometry, to form C and the species C and B reversibly combine, with a 1:1 stoichiometry, to form D.

ASSUME that:

1. The total, initial, concentration of A is A_0 and that A_0 is the same for all data points in a given experiment/test.
2. The total concentration of B, B_0 , changes from one data point to the next.
3. Initially, when $B_0 = 0$, the concentrations of C and D are zero. That is, no C or D is present until B has been added to the solution/mixture.

The result of the above is that for any given data point, the concentration of the various species are:

$$[C] = x_1 \quad \dots(3.5)$$

$$[D] = x_2 \quad \dots(3.6)$$

$$[B] = B_0 - x_1 - 2 * x_2 \quad \dots(3.7)$$

$$[A] = A_0 - x_1 - x_2 \quad \dots(3.8)$$

Define the equilibrium quotients, Q_1 and Q_2 , (aka the formation constants) as:

$$Q_1 = [C] / ([A] * [B]) \quad \dots(3.9)$$

$$Q_2 = [D] / ([C] * [B]) \quad \dots(3.10)$$

The Q 's can now be expressed in terms of the total concentrations of A, A_0 , and B, B_0 , that have been admixed in the solution to obtain:

$$Q_1 = x_1 / ((A_0 - x_1 - x_2) * (B_0 - x_1 - 2 * x_2)) \quad \dots(3.11)$$

$$Q_2 = x_2 / (x_1 * (B_0 - x_1 - 2 * x_2)) \quad \dots(3.12)$$

ASSUME that the concentration of B is always very much greater than that of A_0 , C and D; that is, $B_0 \gg (A_0 + x_2)$. Typically this means that B_0 is at least 10 times A_0 plus $[D]$; that is, $B_0 \geq 10 * (A_0 + x_2)$.

Applying the above assumption to eq 11 results in:

$$Q_1 = x_1 / ((A_0 - x_1 - x_2) * B_0) \quad \dots(3.13)$$

or

$$Q_1 * B_0 * A_0 - Q_1 * B_0 * x_1 - Q_1 * B_0 * x_2 = x_1 \quad \dots(3.14)$$

and applying it to eq 12 results in:

$$Q2 = x2 / (x1*Bo) \quad \dots(3.15)$$

or

$$x2 = Q2*Bo*x1 \quad \dots(3.16)$$

Substitute eq 3.16 into eq 3.14 and solve for x1 to obtain:

$$x1 = Q1*Ao*Bo / (1 + Q1*Bo + Q1*Q2*Bo2) \quad \dots(3.17)$$

Substitute eq 3.17 in to eq 3.16 and solve for x2 to obtain:

$$x2 = Q1*Q2*Ao*Bo2 / (1 + Q1*Bo + Q1*Q2*Bo2) \quad \dots(3.18)$$

At this point it is necessary to relate the concentrations of species A, C and D to a physical property that can be (easily) measured.

The measured, species specific, response, R_i , can be a measure of any property the follows Beer's Law; that is, a property that is directly proportional to the concentration of that species. It could, for example, be the molar fluorescence, the molar absorbtivity, the molar conductivity or the reaction rate.

Define the species specific response factors as R_a , R_b , R_c and R_d , respectively, for the species A, B, C and D.

This means that the total measured response, R , is given by:

$$R = R_a*[A] + R_b*[B] + R_c*[C] + R_d*[D] \quad \dots(3.19)$$

ASSUME, as is often the case, that $R_b = 0$.

Applying this assumption means that the total response is given by:

$$R = R_a*[A] + R_b*[B] + R_c*[C] + R_d*[D] \quad \dots(3.20)$$

or

$$R = R_a*(Ao - x1 - x2) + R_c*x1 + R_d*x2 \quad \dots(3.21)$$

or

$$R = R_a*(Ao - x1 - x2) + R_c*x1 + R_d*x2 \quad \dots(3.22)$$

or

$$R = R_a*Ao + (R_c - R_a)*x1 + (R_d - R_a)*x2 \quad \dots(3.23)$$

Substitute the values of $x1$ (eq 3.17) and $x2$ (eq 3.18) into eq 3.22 and use the common denominator $(1 + Q1*Bo + Q1*Q2*Bo^2)$ to obtain:

$$R = (R_a*Ao + R_c*Ao*Q1*Bo + R_d*Ao*Q1*Q2*Bo2) / (1 + Q1*Bo + Q1*Q2*Bo2) \quad \dots(3.24)$$

Let

$$R_o = R_1a*A1o + R_2a*A2o; \text{ that is the response when } Bo = 0. \quad \dots(3.25)$$

$$R_{inf} = R_d*Ao; \text{ that is the response when } Bo = \text{infinity}, \quad \dots(3.26)$$

$$\Delta R_1 = R_c*Ao \quad \dots(3.27)$$

$$\Delta R_2 = R_d*Ao \quad \dots(3.28)$$

Then

$$R = (R_o + \Delta R_1*Q1*Bo + \Delta R_2*Q1*Q2*Bo2) / (1 + Q1*Bo) + \Delta R_2*Q2*Bo/(1 + Q2*Bo) \quad \dots(3.29)$$

Additional Notes

1. This is the same as **FitAll** Research Edition's function 0203 with two coupled binding curves, in which

$$\begin{aligned} Y &= R \\ X &= B_0 \\ P1 &= R_0 \\ P2 &= Q1 \\ P3 &= \Delta R1 \\ P4 &= Q2 \\ P5 &= \Delta R2 \end{aligned}$$

2. If it is ASSUMED that $R1a = 0$ and $R2a = 0$ then this is the same as **FitAll** Research Edition's function 0202 with two binding curve, in which

$$\begin{aligned} Y &= R \\ X &= B_0 \\ P1 &= Q1 \\ P2 &= \Delta R1 \\ P3 &= Q2 \\ P4 &= \Delta R2 \end{aligned}$$

3. If there are more than two species/products formed the resulting equation will have a similar form, but have more parameters, P, to resolve.

4. Co-operative Binding/Saturation Curve

The following shows how the fitting function is derived (arrived at) for a single co-operative binding system.

The system to be described by the “n” reversible equilibrium equations/reactions:



and



and

...

and



The basic/fundamental assumption in “co-operative binding” is that each successive binding (formation) constant for the binding of B to A or B to AB, etc. is much larger than the previous one.

The result of this assumption is that there are only three species, A, B and AB_n , in the solution at any given time. That is, intermediate species are not present.

Therefore, the reversible equilibrium equation can be simplified to:



; that is, the species A and B reversibly combine, with a 1: n stoichiometry, to form C

ASSUME that:

1. The total, initial, concentration of A is A_0 and that A_0 is the same for all data points in a given experiment/test.
2. The total concentration of B, B_0 , changes from one data point to the next.
3. Initially, when $B_0 = 0$, the concentration of C is zero. That is, no C is present until B has been added to the solution/mixture.

The result of the above is that for any given data point, the concentration of the various species are:

$$[C] = x \quad \dots(4.5)$$

$$[B] = B_0 - n \cdot x \quad \dots(4.6)$$

$$[A] = A_0 - x \quad \dots(4.7)$$

Define the equilibrium quotients, Q (aka the formation constant) as:

$$Q_1 = [C] / ([A] \cdot [B]^n) \quad \dots(4.8)$$

Q can now be expressed in terms of the total concentrations of A, A_0 , and B, B_0 , that have been admixed in the solution to obtain:

$$Q = x / ((A_0 - x) \cdot (B_0 - n \cdot x)^n) \quad \dots(4.9)$$

ASSUME that the concentration of B is always very much greater than that of A_0 and C; that is, $B_0 \gg (A_0 + n \cdot x)$. Typically this means that B_0 is at least 10 times A_0 ; that is, $B_0 \geq 10 \cdot A_0$.

Applying the above assumption to eq 9 results in:

$$Q = x / ((A_0 - x) \cdot B_0^n) \quad \dots(4.10)$$

or

$$Q \cdot A_o \cdot B_o - Q \cdot B_o \cdot x = x \quad \dots(4.11)$$

Therefore,

$$x = Q \cdot A_o \cdot B_o / (1 + Q \cdot B_o) \quad \dots(4.12)$$

At this point it is necessary to relate the concentrations of species A, B and C to a physical property that can be (easily) measured.

The measured, species specific, response, R_i , can be a measure of any property that follows Beer's Law; that is, a property that is directly proportional to the concentration of that species. It could, for example, be the molar fluorescence, the molar absorptivity, the molar conductivity or the reaction rate.

Define the species specific response factors as R_a , R_b , and R_c , respectively, for the species A, B, and C.

This means that the total measured response, R , is given by:

$$R = R_a \cdot [A] + R_b \cdot [B] + R_c \cdot [C] \quad \dots(4.13)$$

ASSUME, as is often the case, that $R_b = 0$.

Applying this assumption means that the total response is given by:

$$R = R_a \cdot [A] + R_c \cdot [C] \quad \dots(4.14)$$

or

$$R = R_a \cdot (A_o - x) + R_c \cdot x \quad \dots(4.15)$$

or

$$R = R_a \cdot A_o + (R_c - R_a) \cdot x \quad \dots(4.16)$$

Substitute the value of x (eq 4.12) into eq 4.16 to obtain:

$$R = R_a \cdot A_o + (R_c - R_a) \cdot A_o \cdot Q \cdot B_o / (1 + Q \cdot B_o) \quad \dots(4.17)$$

which is the same as

$$R = (R_a \cdot A_o + R_c \cdot A_o \cdot Q \cdot B_o) / (1 + Q \cdot B_o) \quad \dots(4.18)$$

Let

$$R_o = R_a \cdot A_o; \text{ that is the response when } B_o = 0. \quad \dots(4.19)$$

$$R_{inf} = R_c \cdot A_o; \text{ that is the response when } B_o = \text{infinity}, \quad \dots(4.20)$$

Then

$$R = (R_o + R_{inf} \cdot Q \cdot B_o) / (1 + Q \cdot B_o) \quad \dots(4.21)$$

Additional Notes

1. This is the same as *FitAll* Research Edition's function 0204, in which

Y = R
X = B_o
P1 = Q
P2 = R_{inf}
P3 = R_o
P4 = n