Chemical Examples for the Fit Equations

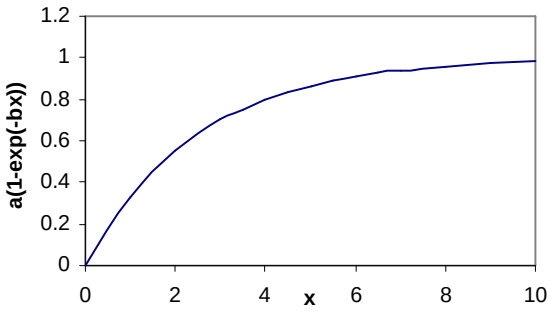
The following are just a few of the applications for nonlinear curve fitting. A nice introduction to biological applications of nonlinear curve fitting by Dr. Harvey Motulsky is available on the Web[1].

a exp(-bx) $f = a\, e^{-bx}$

Example: Chemical kinetics first order decay of a reactant.

$A \rightarrow P$ gives $[A] = [A]_o\, e^{-kt}$

a(1-exp(-bx)) $f = a\,(1 - e^{-bx})$



Example: Chemical kinetics first order increase of a product.

$A \rightarrow P$ gives $[P] = [P]_o(1 - e^{-kt})$

a(1-exp(-b(x-c))) $f = a\,(1 - e^{-b(x-c)})$

Example: This form is the same function as above with an x axis offset. For first order kinetics a delay in the time of the start of the reaction of t_o would give the rate law

$[P] = [P]_o(1 - e^{-k(t-t_o)})$ where $t_o = c$ in the fitting function.

a(1-exp(-bx)) + c $f = a\,(1 - e^{-bx}) + c$

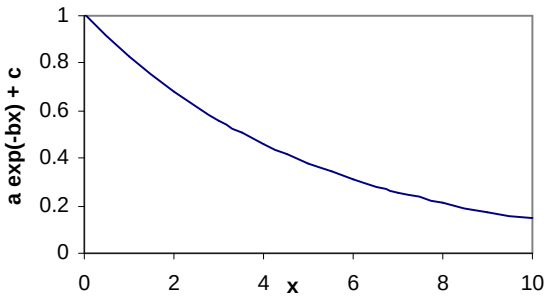
Example: This form introduces a constant offset for first order type growth. For a first order chemical reaction

$[P] = [P]_o(1 - e^{-kt}) + c$

where the offset, c, is due to miscalibration or an instrumental artifact.

This form can be used interchangeably with $f = A\, e^{-bx} + C$ as given in the next function with $A = -a$ and $C = a+c$.

a exp(-bx) + c $f = a\, e^{-bx} + c$



Example: Chemical kinetics first order decay of a reactant towards equilibrium.

$A \underset{\leftarrow}{\overset{\rightarrow}{}} P$ with k_1 the forward rate constant and k_{-1} the reverse rate constant

The linear form for the concentration of the reactant in a reversible first order reaction is

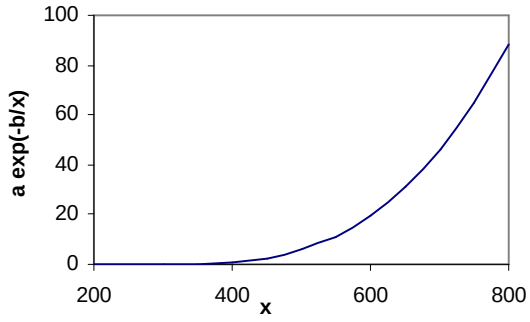
$$\ln([A]-[A]_{eq}) = -(k_1 + k_{-1})\, t + \ln([A]_o-[A]_{eq})$$

which rearranges to give:

$$[A] = ([A]_o-[A]_{eq})\, e^{-(k_1 + k_{-1})t} + [A]_{eq}$$

Comparing to "a exp(-bx) + c" gives $a = ([A]_o-[A]_{eq})$ and $b = k_1 + k_{-1}$ and $c = [A]_{eq}$.

a exp(b/x) + c $f = a\, e^{-b/x} + c$



Example 1: Gibb's Free energy relationships.

Starting from the equation $\Delta_r G^{\ominus} = -RT \ln K$ then $K = e^{-\Delta_r G^{\ominus}/RT}$

or $K = e^{-\Delta_r S^{\ominus}/R}\, e^{-\Delta_r H^{\ominus}/RT}$

Comparing to "a exp(b/x) + c" gives $a = e^{-\Delta_r S^{\ominus}/R}$ and $b = -\Delta_r H^{\ominus}/R$ and c should be fixed at 0.

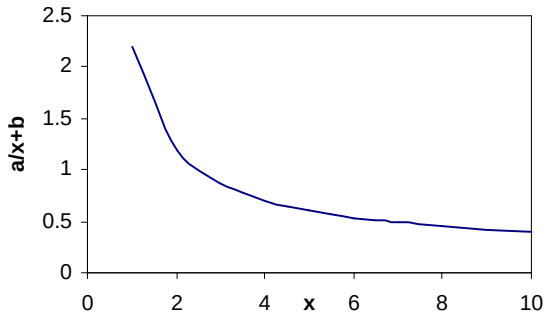
Example 2: Arrhenius temperature dependence in chemical kinetics.

The temperature dependence of a second order rate constant is often given by $k_2 = A\, e^{-E_a/RT}$.

ax^2 + bx + c $f = a\, x^2 + b\, x + c$

Example: Power series are a generally useful functional form, especially when you don't know which function to use. For example the enthalpy of a chemical reaction can be expanded in a power series in the temperature: $\Delta_r H_T = \Delta_r H_o + \Delta a T + \frac{\Delta b}{2} T^2$.

a/x + b $f = \frac{a}{x} + b = \frac{a + bx}{x}$



Example: Michaelis - Menten Enzyme kinetics, Scatchard experiments, and binding isotherms are often rearranged to give this form[2]. Please see the ax/(b+x) section below for a general introduction. Starting with

$$F = \frac{A\,x}{B + x}$$

Inverting gives

$$\frac{1}{F} = \frac{B + x}{A\,x} = \frac{B/A}{x} + \frac{1}{A} = \frac{a}{x} + b \qquad \text{where } a = B/A \text{ and } b = 1/A$$

Then a plot of 1/F verses 1/x gives a straight line. Explicitly for Michaelis - Menten enzyme kinetics:

$$1/\text{rate} = \frac{k_M}{V_{max}\,[S]} + 1/V_{max}$$

This plot for enzyme kinetics is called the Lineweaver-Burk plot, and for binding experiments is called the Benesi-Hildebrand plot. This linear plot is also called a *double-reciprocal plot*. The difficulty with this linear form is that experimental error in the data points isn't constant over the plot. This makes the linear curve hard to fit unless appropriate weighing factors for the errors in the x and y-values are used. Since important parameters are determined from 1/intercept of the plot, the errors in the curve fitting translates to very large errors in the final values.

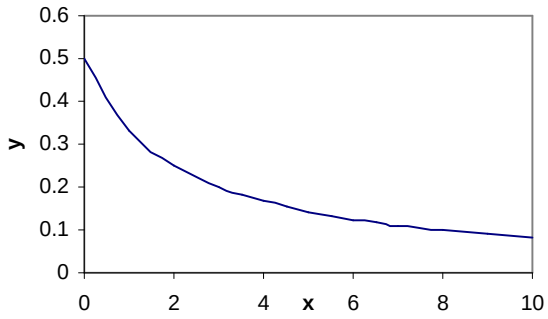
An alternative to the double-reciprocal plot that has better properties for curve fitting is to use the a/x + b form in a nonlinear fit verses x instead of 1/x.

ax + b/x + c $f = ax + \frac{b}{x} + b$

Example: The van Dempter equation, which describes the variation in plate height with flow rate in column chromatography uses this basic form, with x the linear flow rate through the column.

1/(ax+b)

$$f = \frac{1}{a\,x + b}$$



Example: Chemical kinetics second order decay of a reactant.

$2A \rightarrow P$ with k_2 the rate constant

The linear form for the concentration of the reactant in a second order reaction is

$$\frac{1}{[A]} - \frac{1}{[A]_o} = k_2 t$$

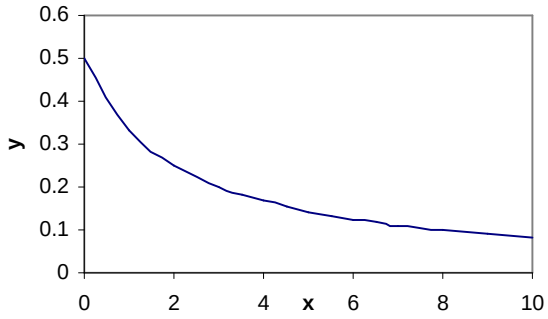
Solving for [A] gives

$$[A] = \frac{1}{k_2 t + 1/[A]_o}$$

Comparing with "1/(ax+b)" gives a = k_2 and b = $1/[A]_o$ when x = time. The next function is more direct and specific for second order kinetics, however.

1/(1/a+bx)

$$f = \frac{1}{1/a + b\,x}$$



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$$\frac{1}{[A]} - \frac{1}{[A]_o} = k_2 t$$

Solving for [A] gives

$$[A] = \frac{1}{1/[A]_o + k_2 t}$$

Comparing with "1/(1/a+bx)" gives a = $[A]_o$ and b = k_2 when x = time.

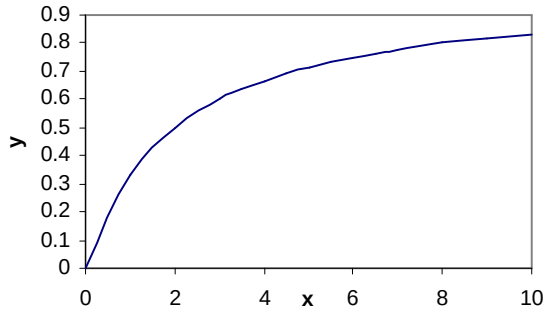
1/(1/a+bx) + c $$f = \frac{1}{1/a + b\,x} + c$$

Example: This form introduces a constant offset for second order type kinetics. For a second order chemical reaction

$$[A] = \frac{1}{1/[A]_o + k_2 t} + c$$

where the offset, c, is due to miscalibration or an instrumental artifact.

ax/(b+x) $$f = \frac{a\,x}{b + x}$$



Example 1: This general form occurs often. Examples include Michaelis - Menten Enzyme kinetics:

$$\text{Rate} = \frac{d[P]}{dt} = \frac{V_{max}\,[S]}{(k_M + [S])} \quad \text{with } k_M = \frac{(k_1 + k_{-1})}{k_2} \text{ and } V_{max} = k_1[E]_o$$

It is often better to fit to the nonlinear form above than the linearized forms such as the Lineweaver-Burk form.

Example 2: Scatchard experiments for 1:1 binding also have the same form.
A typical Scatchard analysis gives

$$K = \frac{\nu}{(1 - \nu)[A]_{free}} \quad \text{with } \nu = [A]_{bound} / [M],$$

where [M] is the concentration of binding sites, $[A]_{bound}$ is the concentration of ligand bound, and [M] is the maximum concentration of binding sites. ($[A]_{bound}$ can also be written [AM] for $A + M \rightleftarrows AM$.) The fraction of bound sites is ν. Rearranging to solve for ν gives:

$$\nu = \frac{K\,[A]_{free}}{1+K[A]_{free}} \quad \text{and dividing by K gives } \nu = \frac{[A]_{free}}{1/K+[A]_{free}}$$

Scatchard type binding experiments are sometimes called saturation binding[1] or specific binding experiments. In this context Y = specific binding and

$$Y = \frac{B_{max}\,X}{K_d + X}$$

where X is the ligand concentration, Bmax is the maximum binding capacity and K_d is the equilibrium constant.

Example 3: The Langmuir isotherm from surface chemistry is given by

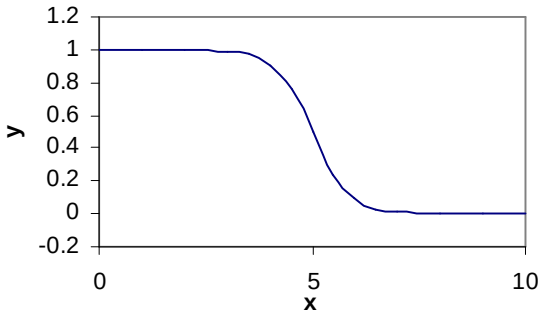
$$\theta = \frac{K\,p}{(1 + K\,p)}$$ where θ is the surface coverage and p is the pressure.

ax/(b+x) + cx $$f = \frac{a\,x}{b + x} + cx$$

Example: Nonspecific binding is directly proportional to the ligand concentration, Y = Ns X. Where Ns is a measure of the nonspecific binding capacity of the enzyme or host. Combining specific and nonspecific binding (see "ax/(b+x)") gives the form[1]:

$$Y = \frac{B_{max}\,X}{K_d + X} + Ns\,X$$

a/(1+10^(x-b)) + c $$f = \frac{a}{1 + 10^{(x-b)}} + c$$



Example: Competitive binding experiments in biological or pharmaceutical analysis.

This type of curve is called a "sigmoid dose response curve." In the drug binding context assume A and B compete for a given enzyme binding site:

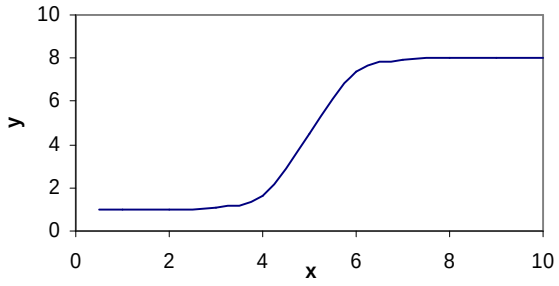
$$[B] = [B]_{min} + \frac{[B]_{max}-[B]_{min}}{1 + 10^{(x-logEC50)}}$$

where [B] is the amount of bound ligand, $[B]_{max}$ is the maximum bound, $[B]_{min}$ is the minimum bound, x = log[A], and logEC50 is the log concentration of A that displaces 50% of B. logEC50% values are commonly quoted in the literature. Comparing to the form "a/(1+10^(x-b)) + c", a = $[B]_{max}$-$[B]_{min}$, b = logEC50, and c = $[B]_{min}$.

This form assumes a standard slope, giving a 10% to 90% response for a change in drug concentration of about two orders of magnitude (assuming x = log[A]).

a/(1+10^(b-x)) +c

$$f = \frac{a}{1 + 10^{(b-x)}} + c$$



Example: Binding experiments in biological or pharmaceutical analysis.

This type of curve is called a "sigmoid dose response curve," where the response increases with the dose. The form given above (see "a/(1+10^(x-b))+c") with (x-b) gives a decreasing response with dose. In the drug binding context:

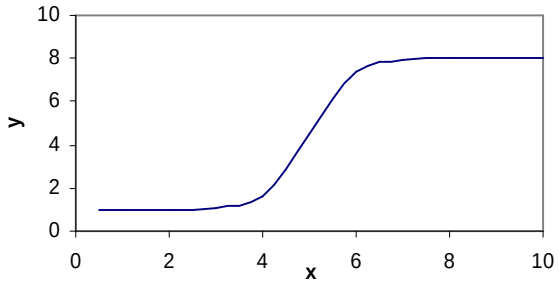
$$Y = [A]_{min} + \frac{[A]_{max}-[A]_{min}}{1 + 10^{(logEC50-x)}}$$

Where $Y = [A]_{bound}$, or the function value can be a pharmacological result. In dose response experiments x = log[A], where [A] is the free ligand concentration. Again comparing to the form "a/(1+10^(b-x)) + c", $a = [A]_{max}-[A]_{min}$, b = logEC50, and $c = [A]_{min}$. The EC50 value is the effective concentration for 50% response. The EC50 value is sometimes called ED50, for effective dose, or IC50, for inhibitory concentration.

This form assumes a standard slope, giving a 10% to 90% response for a change in drug concentration of about two orders of magnitude (assuming x = log[A]).

(a 10^(x-b) + c)/(1+10^(x-b))

$$f = \frac{a\ 10^{(x-b)} + c}{1+10^{(x-b)}}$$



This form and the previous form are very similar. The difference is how the extremes are expressed. In this form the a parameter is the maximum value, and not max-min. The b and c parameters remain the same. When x = b, f = (a+c)/2, the average value.

Example: This form works well with binding experiments, as discussed above. This form is also particularly useful in other types of chemical equilibrium experiments. One specific example is the use of pH indicator species in NMR. Assume that a particular species can exist in two forms, $HI \rightleftarrows H^+ + I^-$ with a given pK_a. The limiting chemical shifts of the two forms are δ_{HI} and δ_{I^-}. The observed chemical shift is given by the mole fraction weighted average:

$$\delta_{obs} = \frac{[HI]}{[HI]+[I^-]}\delta_{HI} + \frac{[I^-]}{[HI]+[I^-]}\delta_{I^-}$$

Defining the mole fraction of I^- as

$$X = \frac{[I^-]}{[HI]+[I^-]} = \frac{[I^-]}{[HI]_t}$$

and noting that $X_{HI} = 1 - X$ gives:

$$\delta_{obs} = (1\text{-}X)\,\delta_{HI} + (X)\,\delta_{I^-}$$

The pH of the solution is given by the Henderson-Hasselbach equation

$$pH = pK_a + \log\left(\frac{[A^-]}{[HI]}\right)$$

The concentration ratio is the same as the mole fraction ratio:

$$\left(\frac{[A^-]}{[HI]}\right) = \left(\frac{X}{1\text{-}X}\right)$$

Solving for this ratio from $\delta_{obs} = (1\text{-}X)\,\delta_{HI} + (X)\,\delta_{I^-}$ gives

$$X = \left(\frac{\delta_{obs} - \delta_{HI}}{\delta_{I^-} - \delta_{HI}}\right) \qquad 1\text{-}X = \left(\frac{\delta_{I^-} - \delta_{obs}}{\delta_{I^-} - \delta_{HI}}\right) \quad \text{and}$$

$$\left(\frac{[A^-]}{[HI]}\right) = \left(\frac{X}{1\text{-}X}\right) = \left(\frac{\delta_{obs} - \delta_{HI}}{\delta_{I^-} - \delta_{obs}}\right)$$

Substituting into the Henderson Hasselbach equation then gives

$$pH = pK_a + \log\left(\frac{\delta_{obs} - \delta_{HI}}{\delta_{I^-} - \delta_{obs}}\right)$$

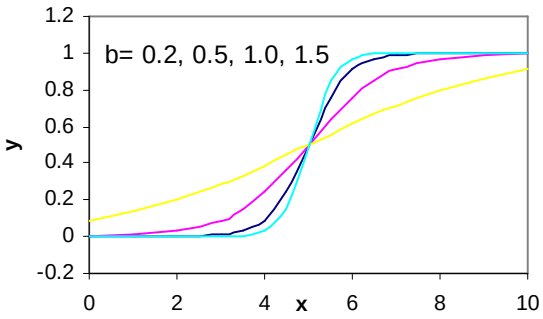
This functional form is not useful for curve fitting. The plot of the chemical shift versus the pH is more amenable to curve fitting. So solving this equation for δ_{obs} as a function of pH gives:

$$\delta_{obs} = \frac{\delta_{I^-}\,10^{(pH-\,pKa)} + \delta_{HI}}{1+10^{(pH-\,pKa)}}$$

As the pH increases, the shift changes from δ_{HI} to δ_{I^-} in a sigmoidal fashion. When $pH = pK_a$, $[HI] = [I^-]$, and the shift is the normal average of the two forms. Comparing to the curve fit functional form, $a = \delta_{I^-} = \delta_{max}$, $c = \delta_{HI} = \delta_{min}$, and $b = pK_a$. Once the curve fit parameters are determined, they can be substitutued into the Henderson-Hasselbach equation to calculate the pH of an unknown. This type of relationship should hold whenever the observed response is mole fraction weighted.

a/(1+10^(b(c-x)))

$$f = \frac{a}{1 + 10^{b(c-x)}}$$

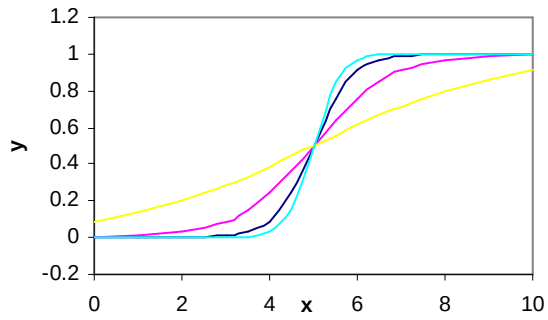


Example: Sigmoid dose response curve with a variable slope.

Since this form, a/(1+10^(b(c-x))) doesn't have the "B_{min}" constant term you will need to subtract out the $[B]_{min}$ before you can do the curve fit. The b constant is the variable slope, which is called the Hill slope. When b is less than one, the curve is shallower. When b is greater than 1 the curve changes more sharply. When b = 1, the curve follows the standard slope (see discussions above).

a/(1+exp(b(c-x)))

$$f = \frac{a}{1 + e^{b(c-x)}}$$



Example: Sigmoid dose response curve with a variable slope.

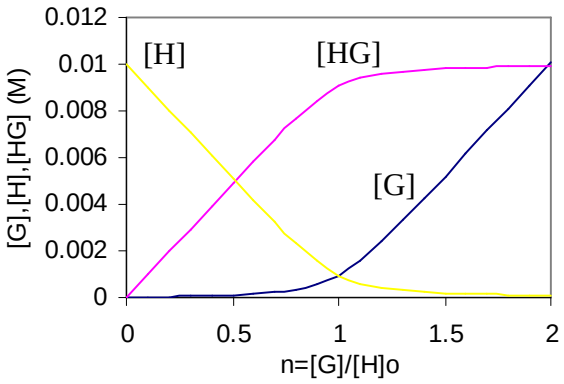
Note that you can convert between the base 10 and base e forms of these equations since $10^x = e^{2.303x}$. Then $e^{b(c-x)} = 10^{B(C-x)} = e^{2.303B(C-x)}$ and taking the log of both sides gives:

$$b(c-x) = 2.303\, B(C-x)$$

and comparing terms gives b = 2.303 B and c = C .

a{-(1+b(1-x))+sqrt[(1+b(1-x))^2+4bx]}/2b

$$f = a\,\frac{-(1+b(1-x)) + \sqrt{(1+b(1-x))^2 + 4bx}}{2b}$$



Example: Chemical equilibrium:

Guest-host binding:	$G + H \rightleftarrows [HG]$
Weak base equilibria:	$H^+ + B \rightleftarrows HB^+$
Weak acid equilibria:	$H^+ + A^- \rightleftarrows HA$

Focusing on guest-host equilibria: $G + H \rightleftarrows HG$

Assume that you have a solution with a total concentration of guest, $[G]_t$, and host, $[H]_t$, then the mass balance equations are:

$$[G]_t = [G] + [HG]$$
$$[H]_t = [H] + [HG]$$

then the free guest concentration is given by[3]:

$$[G] = \frac{-(1+K([H]_t-[G]_t)) + \sqrt{(1+K([H]_t-[G]_t))^2 + 4K[G]_t}}{2K}$$

The mole ratio of the added guest to host is $n = \frac{[G]_t}{[H]_t}$. Multiplying and dividing the equilibrium constant by $[H]_t$ gives:

$$[G] = \frac{-(1+K[H]_t\,(1-n)) + \sqrt{(1+K[H]_t\,(1-n))^2 + 4K[H]_t\,n}}{2K[H]_t/[H]_t}$$

Or

$$[G] = [H]_t\left(\frac{-(1+K[H]_t\,(1-n)) + \sqrt{(1+K[H]_t\,(1-n))^2 + 4K[H]_t\,n}}{2K[H]_t}\right)$$

The mole balance equations can be used to calculate [HG] and [H], or the equilibrium expressions can be solved directly giving:

$$[H] = [H]_t\left(\frac{-(1+K[H]_t\,(n-1)) + \sqrt{(1+K[H]_t\,(n-1))^2 + 4K[H]_t}}{2K[H]_t}\right)$$

$$[HG] = [H]_t\left(\frac{(1+K[H]_t\,(1+n)) - \sqrt{(1+K[H]_t\,(1+n))^2 - 4K^2[H]_t^2\,n}}{2K[H]_t}\right)$$

The equations are in the form:

$$[G] = a\,\frac{-(1+b(1-x)) + \sqrt{(1+b(1-x))^2 + 4bx}}{2b}$$

$$[H] = a\left(\frac{-(1+b(x-1)) + \sqrt{(1+b(x-1))^2 + 4b}}{2b}\right)$$

$$[HG] = a\left(\frac{(1+b(1+x)) - \sqrt{(1+b(1+x))^2 - 4b^2\,x}}{2b}\right)$$

with $a = [H]_t$ and $b = K[H]_t$ with $x = n$. The fractional occupation $\nu=[H]/[H]_t$ can also be calculated from the equation for [H]:

$$\nu = \left(\frac{-(1+b(x-1)) + \sqrt{(1+b(x-1))^2 + 4b}}{2b}\right)$$

In actual practice, an experimental response proportional to the concentration may be determined (e.g. the absorbance of the solution), $R = k\,[G]$ and then

$$R = k[G] = a\,\frac{-(1+b(1-x)) + \sqrt{(1+b(1-x))^2 + 4bx}}{2b}$$

Where $a = k[H]_t$. A curve fit of the experimental response verses the mole ratio then gives the equilibrium constant from $b = K[H]_t$. The designation of the guest and host in these equations is arbitrary, so the G and H labels may be exchanged.

Another specific example is the determination of equilibrium constants in NMR in the extreme narrowing limit. For host-guest association, consider $G + H \rightleftarrows HG$ with a given K. The limiting chemical shifts of the three forms are δ_H, δ_G, and δ_{HG}. Assume that the total host concentration is $[H]_t$ and the guest is added in varying amounts, as above. The observed chemical shift of the host is given by the mole fraction weighted average:

$$\delta_{obs} = \frac{[H]}{[H]+[HG]}\,\delta_H + \frac{[HG]}{[H]+[HG]}\,\delta_{HG}$$

Defining the mole fraction of host and host guest complex as

$$X_H = \frac{[H]}{[H]+[HG]} = \frac{[H]}{[H]_t} \qquad X_{HG} = \frac{[HG]}{[H]+[HG]} = \frac{[HG]}{[H]_t}$$

and noting that $X_H = 1 - X_{HG}$ gives:

$$\delta_{obs} = (1-X_{HG})\,\delta_H + (X_{HG})\,\delta_{HG}$$

The observed chemical shift can also be directly related to the host guest complex concentration:

$$\delta_{obs} = \delta_H + (\delta_{HG} - \delta_H)\frac{[HG]}{[H]_t}$$

The $[HG]/[H]_t$ ratio is given by the equation above. The final fitting function is then

$$\delta_{obs} = a\left(\frac{(1+b(1+x)) - \sqrt{(1+b(1+x))^2 - 4b^2 x}}{2b}\right) + c$$

with $a = (\delta_{HG} - \delta_H)$, $b = K[H]_t$.as before, and $c = \delta_H$.

The same equations can easily be used for the other types of chemical equilibria mentioned above[3]. For example for acid-base equilibria the [G] is replaced by $[H^+]$.

As presented here, these equations cannot be used for fitting titration curves, because the total volume increases in a standard titration and $[H]_t$ or its equivalent will not be constant. However, if the dilution factor during the titration is small or the experiment is done at constant total volume, then these equations will be useful.

Nonlinear least squares curve fitting programs available on the Web:

The "Nonlinear Least Squares Curve Fitter" written by John C. Pezzullo will be very useful if the equation form that you want to use is not available in standard packages. In this applet, you can input any functional form:

http://statpages.org/nonlin.html

Another example for several simple equation forms is:

http://people.hofstra.edu/faculty/Stefan_Waner/newgraph/regressionframes.html

Literature Cited:

1. http://curvefit.com/analyzing_data_book.htm
2. K. A. Connors, Binding Constants, The Measurement of Molecular Complex Stability, John Wiley & Sons, New York, NY, 1987; pp. 50-65.
3. D. J. Eatough, J. J. Christensen, R. M. Izatt, Experiments in Thermometric Titrimetry and Titration Calorimetry, Brigham Young University Press, Provo, Utah, 1974; p. 25.