Simulation using Newton-Raphson Method for a Hypothetical Host: Guest = 1 : 2 Complex with a Vertical Plane of Symmetry

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ABSTRACT

We formulate a derivation of host : guest = 1 : N equilibrium model for the purpose of giving an alternative approach to deal with cooperative problems and carefully take a look at three cases of cooperativity factors given three different concentrations. This would take a minor assumption given a hypothetical host-guest complex with a vertical plane of symmetry. These works would help giving an intuition for the researchers who evaluate binding constant and stoichiometry. We also considered Job plots frequently used in supramolecular host-guest chemistry. All simulations using Newton-Raphson method were conducted in simple Python codes.

Understanding the stoichiometry of specific complexes provides a clue to knowledge of complementary effects of intermolecular or allosteric binding and their thermodynamics. Determining a reasonable binding model for a complex is also an essential point. These are usually used to be estimated by fitting physical changes into several models. Over-simplified models do not provide any reliable parameters. From this backdrop, Newton-Raphson method with non-linear regression suggests much closer approximations. We briefly formulate a host : guest = 1 : N equilibrium model to give an option for some specific cases. A hypothetical host-guest complex with a vertical plane of symmetry was used in this case.

On host-guest binding complexes for a host, we usually express first stepwise binding constant as \( K_1 = \frac{[HG]}{[H][G]} \). Mole fraction of HG (\( f_{HG} \)) can be presented by rearranging the equation of first stepwise binding constant (\( K_1 \)) to \([HG] = K_1[G][H] \) and can be divided by \([H]\), both of each side.

\[
f_{HG} = K_1[G]f_H
\]

We can get second stepwise binding constant in the same way,

\[
K_2 = \frac{[HG_2]}{[G][HG]}
\]

After these we can now figure overall binding constant (\( \beta_{12} \)).
\[ K_1K_2 = \frac{[HG_2]}{[G]^2[H]} = \beta_{12} \]

\[ [HG_2] = K_1K_2[G]^2[H] \]

\[ f_{HG_2} = K_1K_2[G]^2f_H \]

In a similar manner, we could generalize these relations below,

\[ K_1K_2 \cdots K_n = \frac{[HG_n]}{[G]^n[H]} = \beta_{1n} \]

\[ [HG_n] = K_1K_2 \cdots K_n[G]^n[H] \]

\[ f_{HG_n} = K_1K_2 \cdots K_n[G]^nf_H \]

Total concentration of host molecule \([H]_t\) is equal to sum of these complexes and free (unsaturated) host \([H]\) respectively,

\[ [H]_t = [H] + [HG] + [HG_2] + \cdots + [HG_n] \]

Above formula can be rearranged by the notation of \([HG_n]\),

\[ [HG_n] = [H]_t - [H] - [HG] - [HG_2] - \cdots - [HG_{n-1}] \]

Both of each sides can be divided by \([H]_t\), we finally get an expression \(f_H\), fraction of unsaturated host,

\[ \frac{[HG_n]}{[H]_t} = \frac{[H]_t - [H] - [HG] - [HG_2] - \cdots - [HG_{n-1}]}{[H]_t} \]

\[ f_{HG_n} = 1 - f_H - f_{HG} + f_{HG_2} + \cdots + f_{HG_n} \]

\[ 1 - f_H = f_{HG} + f_{HG_2} + \cdots + f_{HG_n} \]

1 – \(f_H\) defined as mole fraction of complexes. Substitute each mole fractions of complexes using \(f_{HG_n} = K_1K_2 \cdots K_n[G]^nf_H\) and \(1 - f_H = 1 - \frac{[H]}{[H]_t} = \frac{[H]_t - [H]}{[H]_t}\),

\[ [H]_t = [H] + K_1[G][H] + K_1K_2[G]^2[H] + \cdots + K_1K_2 \cdots K_n[G]^n[H] \]

Both of each sides can be divided by \([H]\),

\[ \frac{[H]_t}{[H]} = 1 + K_1[G] + K_1K_2[G]^2 + \cdots + K_1K_2 \cdots K_n[G]^n = \frac{1}{f_H} \]
By multiplying -1 and adding 1 into both side of the equation,

\[
1 - f_H = \frac{K_1[G] + K_1K_2[G]^2 + \cdots + K_1K_2 \cdots K_n[G]^n}{1 + K_1[G] + K_1K_2[G]^2 + \cdots + K_1K_2 \cdots K_n[G]^n}
\]

\[
\therefore f_{HG} + f_{HG_2} + \cdots + f_{HG_n} = \frac{K_1[G] + K_1K_2[G]^2 + \cdots + K_1K_2 \cdots K_n[G]^n}{1 + K_1[G] + K_1K_2[G]^2 + \cdots + K_1K_2 \cdots K_n[G]^n}
\]

We bring out some simplifications for our convenience. Define \( \alpha \) as a mole fraction of complexes that a single host occupied with guests understandably. Symbol \( v \) also describes a mole fraction of complexes weighted down the number of guests at the complex.

\[
\alpha = \frac{[H]_t - [H]}{[H]_t} = \frac{[HG] + [HG_2]}{[H]_t} = \frac{K_1[G] + K_1K_2[G]^2}{1 + K_1[G] + K_1K_2[G]^2}
\]

\[
v = \frac{[G]_t - [G]}{[H]_t} = \frac{[HG] + 2[HG_2]}{[H]_t} = \frac{K_1[G] + 2K_1K_2[G]^2}{1 + K_1[G] + K_1K_2[G]^2}
\]

Expression \( \alpha \) and \( v \) come up with direct values \( m \) and \( n \) on overall binding constant (\( \beta_{mn} = \frac{[H_mG_n]}{[H]^m[G]^n} \)) through expression \( \frac{v}{\alpha} \) and \( \frac{\alpha}{\alpha + v} \).

\[
[H]_t = [H] + m \cdot [H_mG_n]
\]

\[
\therefore m = \frac{[H]_t - [H]}{[H_mG_n]}
\]

\[
[G]_t = [G] + n \cdot [H_mG_n]
\]

\[
\therefore n = \frac{[G]_t - [G]}{[H_mG_n]}
\]

\[
v = \frac{[G]_t - [G]}{[H]_t - [H]} = \frac{n}{m}
\]

\[
\frac{\alpha}{\alpha + v} = \frac{[H]_t - [H]}{[H]_t - [H] + [G]_t - [G]} = \frac{m}{m + n}
\]

Next, we now carefully take a look at three cases of cooperativity factors given three different concentrations from our host : guest = 1 : N equilibrium model.
Figure 1. Brief diagram of stepwise guest binding equilibrium with a hypothetical host bearing two geometrically equivalent binding sites. (H: host bearing two structurally identical binding sites, G: Guest, HG: first stepwise complexation, HG\(_2\): second stepwise complexation)

A diagram in Figure 1 shows host:guest = 1:2 binding equilibrium. Cooperativity factor (f), an indicator of allosteric effect, is classified as positive (f > 1), none (f = 1) and negative (f < 1). Positive cooperativity factor promotes binding. On the other hand, negative inhibits following coordinations. Non-cooperative means that the first stepwise binding does not affect the second stepwise binding. Relationship of model parameters (K\(_1\), K\(_2\), f) is f = \(\frac{K_2}{K_1}\)^2.

Through the above condition, simulations proceeded to three different cooperativity cases from our model and fitting methodology. Note that the purpose of simulations is to identify the observation of cooperativity’s tendency. For that reason, we assume all overall binding constants are the same.

<table>
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<th></th>
<th>Red (negative-)</th>
<th>Black (none-)</th>
<th>Green (positive-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(f)</td>
<td>0.04</td>
<td>1</td>
<td>400</td>
</tr>
<tr>
<td>K(_1)</td>
<td>100,000</td>
<td>20,000</td>
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</tr>
<tr>
<td>K(_2)</td>
<td>1,000</td>
<td>5,000</td>
<td>100,000</td>
</tr>
</tbody>
</table>

Table 1. Host: Guest = 1:2 binding equilibrium performed by assumed \(f\), K\(_1\), K\(_2\), and host concentration media.
As results of simulation, we visualize each of the three cases (Figure 2). Red, Green and Black lines stand for each case. Red line represents the equilibrium with negative cooperativity \( f = 0.04 \). Black line with non-cooperative \( f = 1 \). Green line with positive cooperativity \( f = 400 \) (Table 1).

When it comes to Figure 2a, the higher first stepwise association \( (K_1) \) precedes a step ahead in the sense of binding saturation. From Figure 2b, though we set the model to be Host : Guest = 1 : 2 binding equilibrium, it hardly reaches \( H : G = 1 : 2 \) stoichiometry in the system. Whereas host has two binding sites for guests, the response of the stoichiometry is insufficient to be \( H : G = 1 : 2 \) given negative and non-cooperative binding equilibrium.

Therefore, we must keep in mind that, even if we already know its structure, obtaining stoichiometry from the titration method is not a good option. From our model, capability of how many guests can be bound is up to its cooperativity at the same guest equivalents.
Figure 3. Visualization plot of stoichiometry ratio \( \frac{m}{m+n} \) given (a) \( f = 0.04 \), (b) \( f = 1 \), and (c) \( f = 400 \) over the course of titration in various host concentration media. The purple dotted line represents the host concentration of 0.00001 M. The blue dotted line represents the host concentration of 0.0001 M. The black dotted line represents the host concentration of 0.001 M. The yellow dotted line represents the host concentration of 0.01 M.

We also conducted simulations by varying the concentration of solution media (Figure 3). In previous, Jurczark\(^5\) already confirmed that the interpretation of Job plots varies with concentration changes. Figure 3 explains host-guest complexation increases corresponding to the number of guest equivalents. It generates most of complexes in the early stages of titrations. Calculated stoichiometry of binding equilibrium is also varied by concentration on the each titration media.

Continuous variation method (Job plot) is mainly one of the analysis methods of stoichiometry. However, according to Jurczark and colleagues\(^5\), while the existing Job plot method is useful for very stable (same as high cooperativity) metal-ligand complexes, the approach in supramolecular chemistry gives incorrect results. And recently, Thordarson and Hibbert assured the end of Job plot in supramolecular chemistry\(^6\). Finally, we conducted Job plot that we re-organized the generated data at above titration simulations.
Figure 4. The host concentration of titration media makes a difference in Job plots illustrated by simulations. *x-coordinate: $\frac{[H]_t}{[H]_t + [G]_t}$, *y-coordinate: $\frac{[H]_t}{[H]_t + [G]_t} \times ([HG] + [HG_2])$

The Job plots in Figure 4 show distortion of the measurement of stoichiometry. The results of Job plot were easily skewed from its initial assumption when experiments were performed at low concentration media. The maximum value of Job plots should deviate from 0.5 depending on its cooperativity given that a host-guest binding equilibrium is host : guest = 1 : 2. But it is too far beyond 0.33 meaning that forming complexes over host : guest = 1 : 2 binding stoichiometry deviates far from our initial assumption. Therefore, our results are consistent with those of other earlier researchers, which means the end of Job plot.$^{5,6}$

In conclusion, we briefly conducted titration simulations of a divalent binding model with a hypothetical host-guest complexation. All simulations using Newton-Raphson method were conducted in simple Python codes. As you can see in these simulations, someone who has a goal for getting host-guest stoichiometry from titration and Job plot would take an unreliable result both in actual and even in simulation environment.
Abbreviations and Symbols

- $f$: cooperativity factor;
- $[H]$: molar concentration of unsaturated host;
- $[G]$: molar concentration of unbound guest;
- $[H]_t$: total concentration of host (unsaturated + saturated);
- $[G]_t$: total concentration of guest (unbound + bound);
- $[H_mG_n]$: total concentration of complexes;
- $K_1$: first stepwise binding constant;
- $K_2$: second stepwise binding constant;
- $m$: overall ratio of the host in complexes;
- $n$: overall ratio of guest in complexes;
- $f_{HG_n}$: mole fraction of $HG_n$;
- $\beta_{mn}$: overall binding constant;
- $\alpha$: mole fraction of complexes;
- $\nu$: mole fraction of complexes weighted on the number of guests;
- $\frac{\nu}{\alpha} = \frac{n}{m}$: mean number of bound guests at complexes;
- $\frac{\alpha}{\alpha+\nu} = \frac{m}{m+n}$: stoichiometry ratio;

References