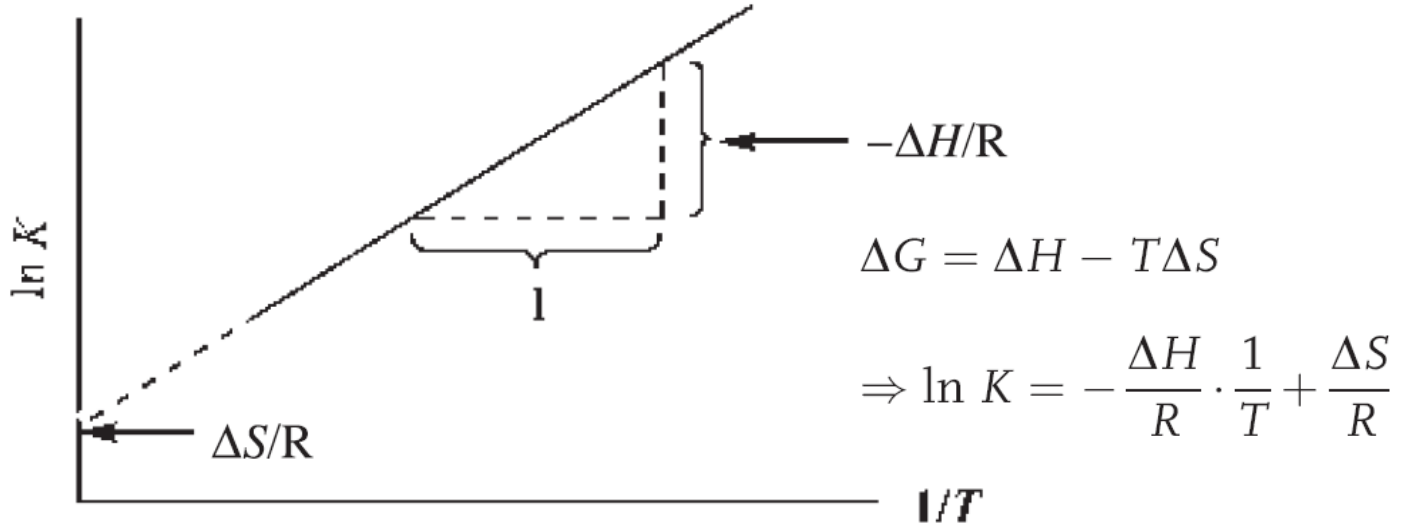
Practical Guide to Determination of Binding Constants



The correlation of ΔH, ΔS, K and temperature according to the van't Hoff equation.

(K. Hirose, in *Analytical Methods in Supramolecular Chemistry*, C. A. Schalley Ed.; Wiley-VCH, Weinheim, 2005)

$$a \cdot \mathrm{H} + b \cdot \mathrm{G} \rightleftarrows \mathrm{C}$$

$$K = \frac{[\mathrm{C}]}{[\mathrm{H}]^a \cdot [\mathrm{G}]^b}$$

$$[\mathrm{H}]_0 = [\mathrm{H}] + a \cdot [\mathrm{C}]$$

$$[\mathrm{G}]_0 = [\mathrm{G}] + b \cdot [\mathrm{C}]$$

$$K = \frac{[\mathrm{C}]}{([\mathrm{H}]_0 - a \cdot [\mathrm{C}])^a \cdot ([\mathrm{G}]_0 - b \cdot [\mathrm{C}])^b}$$

with H: host; G: guest; C: complex ($\mathrm{H}_a \cdot \mathrm{G}_b$)
a, b: stoichiometry as shown in Eq. (2.4)
$[\mathrm{H}]_0$: initial (total) concentration of host molecule
$[\mathrm{G}]_0$: initial (total) concentration of guest molecule
[H], [G], [C]: equilibrium concentrations of host, guest, and complex, respectively

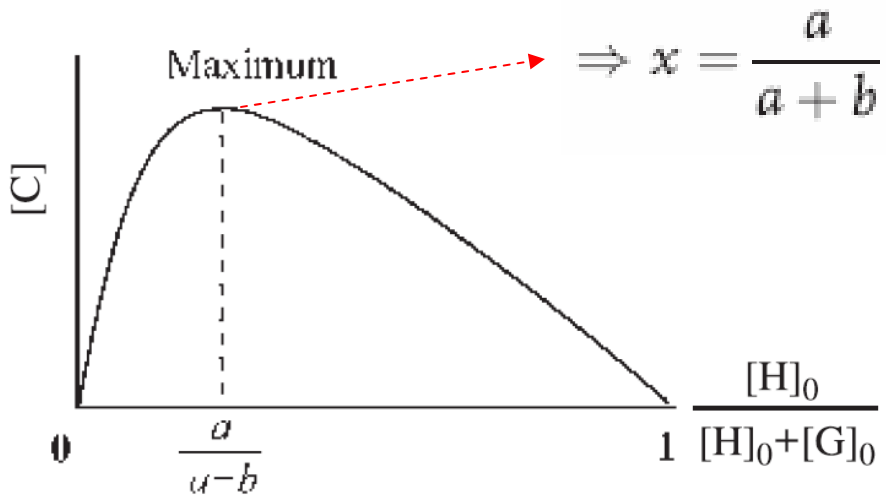
Guideline for Experiments

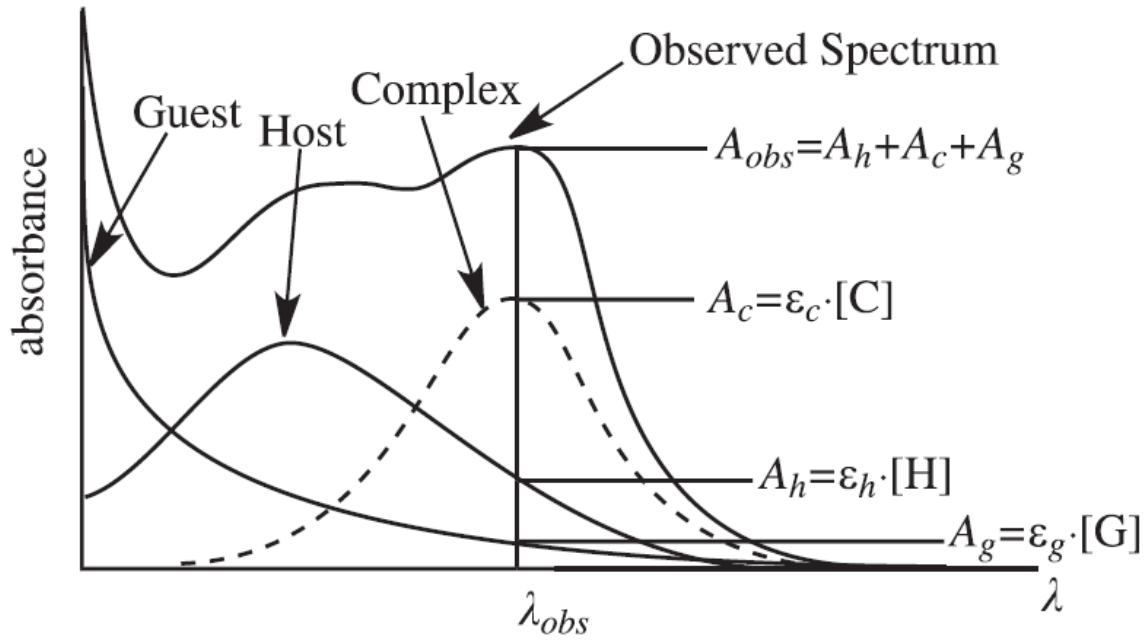
- determination of stoichiometry, namely, a and b
- evaluation of complex concentration, [C]
- setting up the concentration conditions, $[H]_0$ and $[G]_0$
- data treatment.

Determination of Binding Constants by UV-vis Spectroscopy

- Determination of stoichiometry

  - keeping the sum of $[H]_0$ and $[G]_0$ constant (α)
  - changing $[H]_0$ from 0 to α
  - measuring [C]
  - data treatment (Job's plot).





$$A_h = \varepsilon_h \cdot [\mathrm{H}] = \varepsilon_h \cdot ([\mathrm{H}]_0 - a \cdot [\mathrm{C}])$$

$$A_g = \varepsilon_g \cdot [\mathrm{G}] = \varepsilon_g \cdot ([\mathrm{G}]_0 - b \cdot [\mathrm{C}])$$

$$A_c = \varepsilon_c \cdot [\mathrm{C}]$$

$$A_{obs} = A_h + A_g + A_c$$

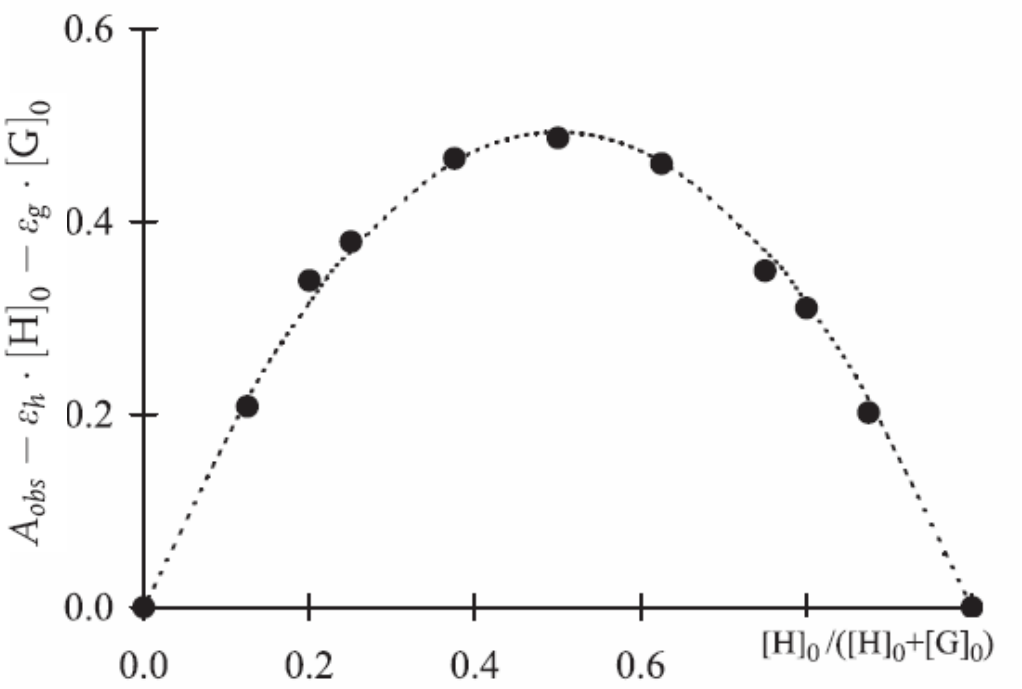
A_{obs}: observed absorbance
A_h, A_g, A_c: absorbances of host, guest, and complex respectively
ε_h, ε_g, ε_c: molar absorptivities of host, guest, and complex respectively

$$A_{obs} = \varepsilon_h \cdot ([\mathrm{H}]_0 - a \cdot [\mathrm{C}]) + \varepsilon_g \cdot ([\mathrm{G}]_0 - b \cdot [\mathrm{C}]) + \varepsilon_c \cdot [\mathrm{C}]$$

$$\Rightarrow A_{obs} - \varepsilon_h \cdot [\mathrm{H}]_0 - \varepsilon_g \cdot [\mathrm{G}]_0 = (\varepsilon_c - a \cdot \varepsilon_h - b \cdot \varepsilon_g) \cdot [\mathrm{C}]$$

The molar absorptivities ε_h, ε_g can be determined from independent measurements using the pure host and the pure guest, respectively. The concentrations $[H]_0$, $[G]_0$, are known because they are the experimental conditions set up by the experimenter.

So, the stoichiometry is determined from a modified Job's plot where (A_{obs} - ε_h $[H]_0$ - ε_g – $[G]_0$ is plotted as the y-coordinate instead of [C].



Modified Job's Plot for complexation of host and guest (1 : 1) by UV/vis spectroscopy.

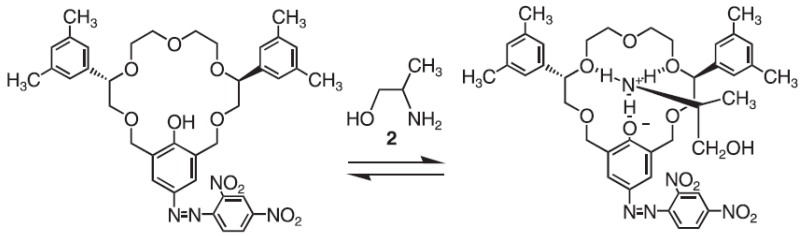
Evaluation of Complex Concentration

- Case 1: the absorption bands of host, guest and complex overlap

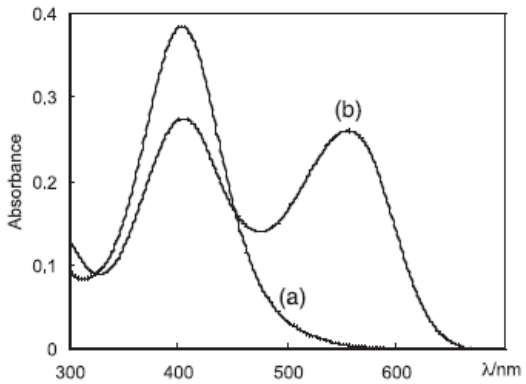
$$[\mathrm{C}] = \frac{A_{obs} - \varepsilon_h \cdot [\mathrm{H}]_0 - \varepsilon_g \cdot [\mathrm{G}]_0}{\varepsilon_c - a \cdot \varepsilon_h - b \cdot \varepsilon_g}$$

- Case 2: the absorption bands of only two components overlap

$$[\mathrm{C}] = \frac{A_{obs} - \varepsilon_h \cdot [\mathrm{H}]_0}{\varepsilon_c - a \cdot \varepsilon_h}$$



Host **1** Complex **3**



(a) (b)

Precautions to be Taken when Setting Up Concentration Conditions of the Titration Experiment

Let us consider 1 : 1 host-guest complexation

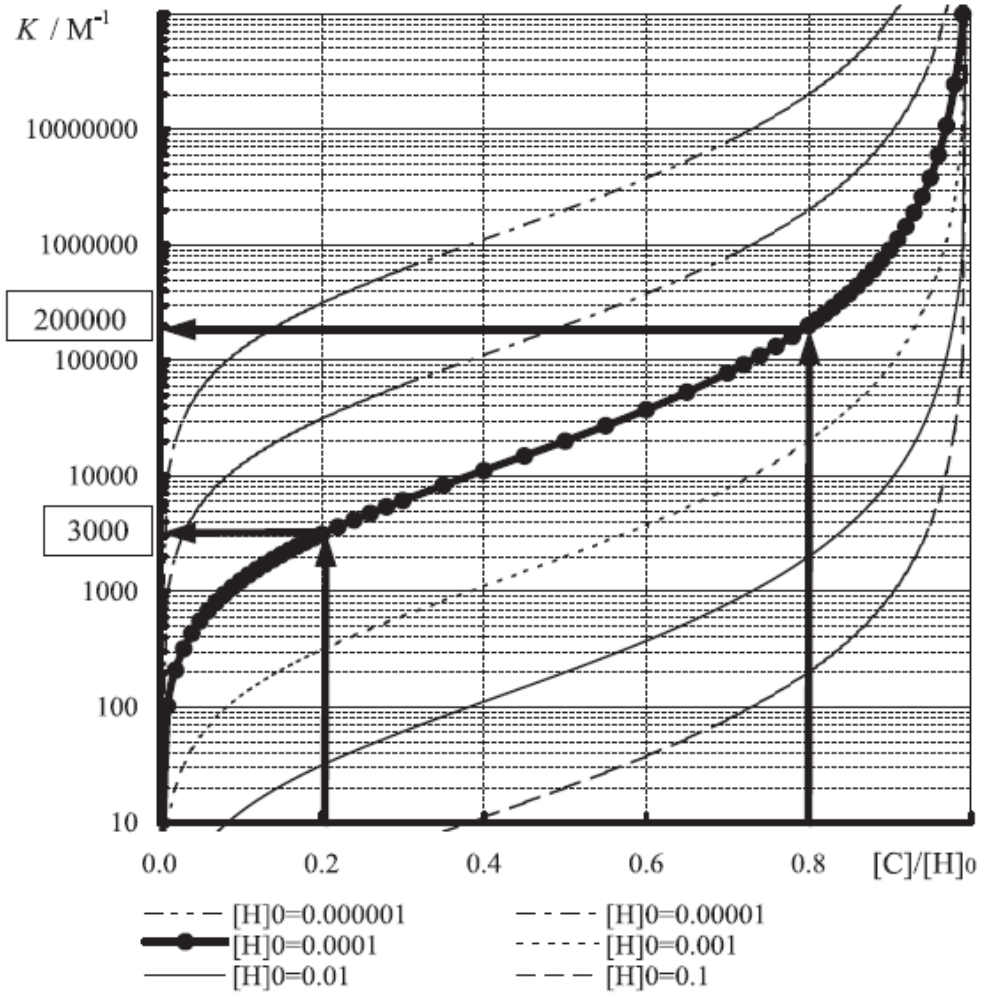
$$\mathrm{H} + \mathrm{G} \rightleftarrows \mathrm{C}$$

$$K = \frac{[\mathrm{C}]}{([\mathrm{H}]_0 - [\mathrm{C}]) \cdot ([\mathrm{G}]_0 - [\mathrm{C}])}$$

$$y = K, \quad x = \frac{[\mathrm{C}]}{[\mathrm{H}]_0}$$

$$y = \frac{x}{(1 - x) \cdot ([\mathrm{G}]_0 - [\mathrm{H}]_0 \cdot x)}$$

$$y = \frac{x}{(1 - x) \cdot ([G]_0 - [H]_0 \cdot x)}$$



Measurements below 20% and above 80% complexation ratio (x) yield uncertain values.

How to Set up $[H]_0$

- Setting up the concentration of host $[H]_0$ is limited by the measured properties, the apparatus, and other features of the experiment.

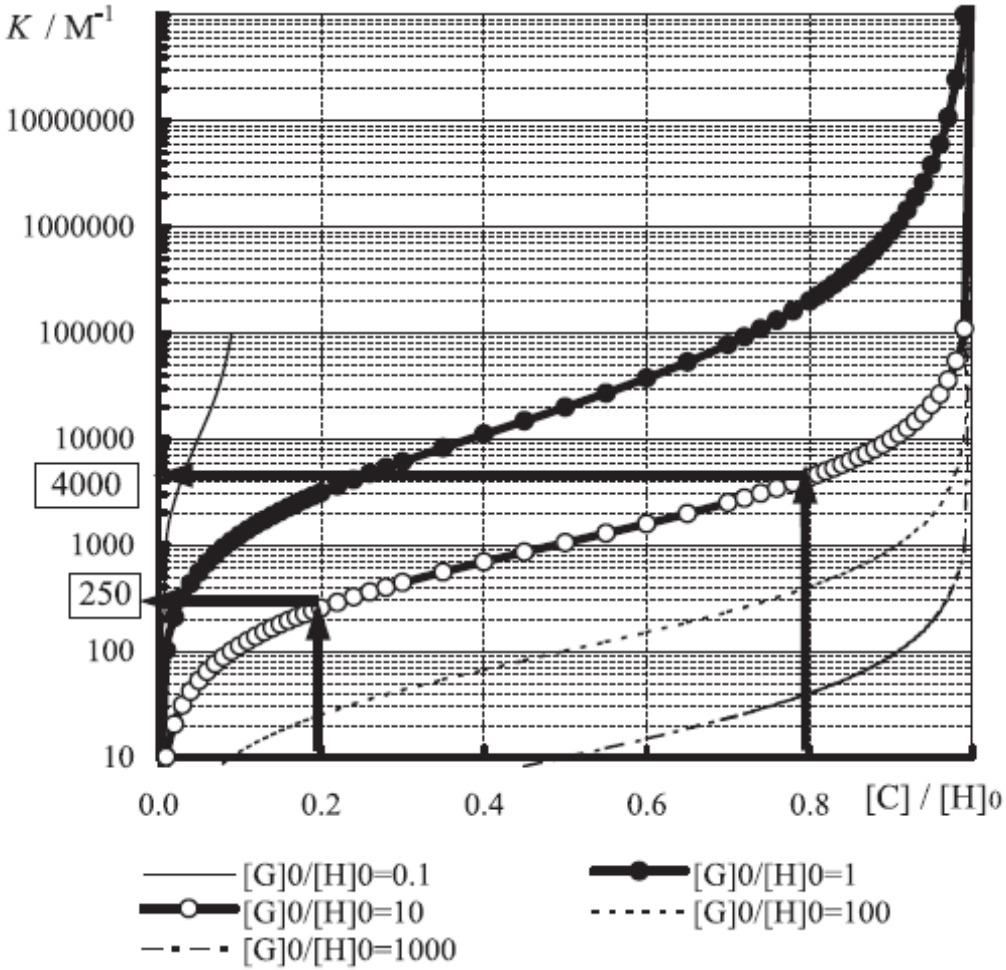
- $[H]_0$ for NMR spectroscopy is roughly in the range of 0.01 M with one or two orders of magnitude variation.

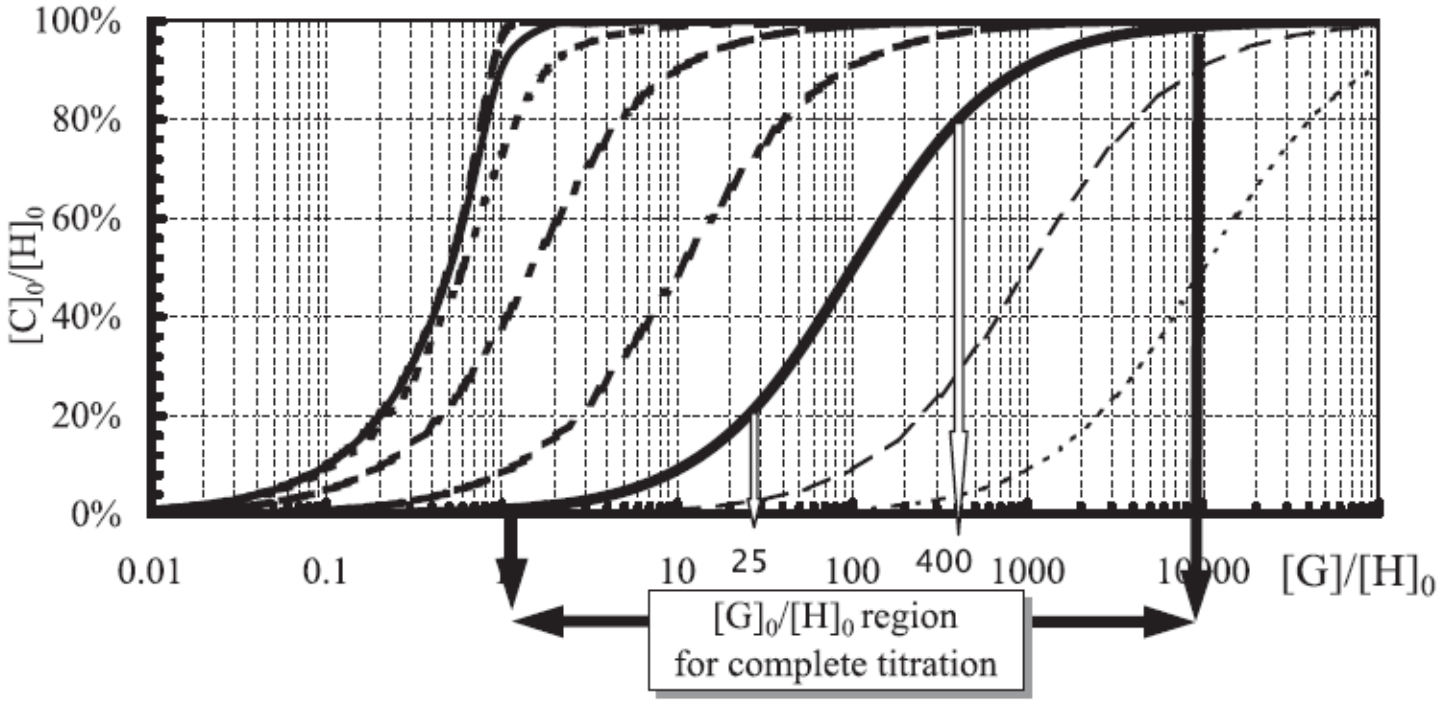
- $[H]_0$ for UV-vis spectroscopy, which depends severely on the molar absorptivity, is roughly in the range of 0.0001 M.

- $[G]_0$ is often the only variable which can be set up in a wide range, because $[H]_0$ is usually governed by the experimental method.

How to Set up $[G]_0$

e.g. when $[G]_0$ = 0.001 M, and $[H]_0$ = 0.0001 M, $[G]_0/[H]_0$ =10, a reliable range of *K* of 250 to 4000 M^{-1} is obtained.



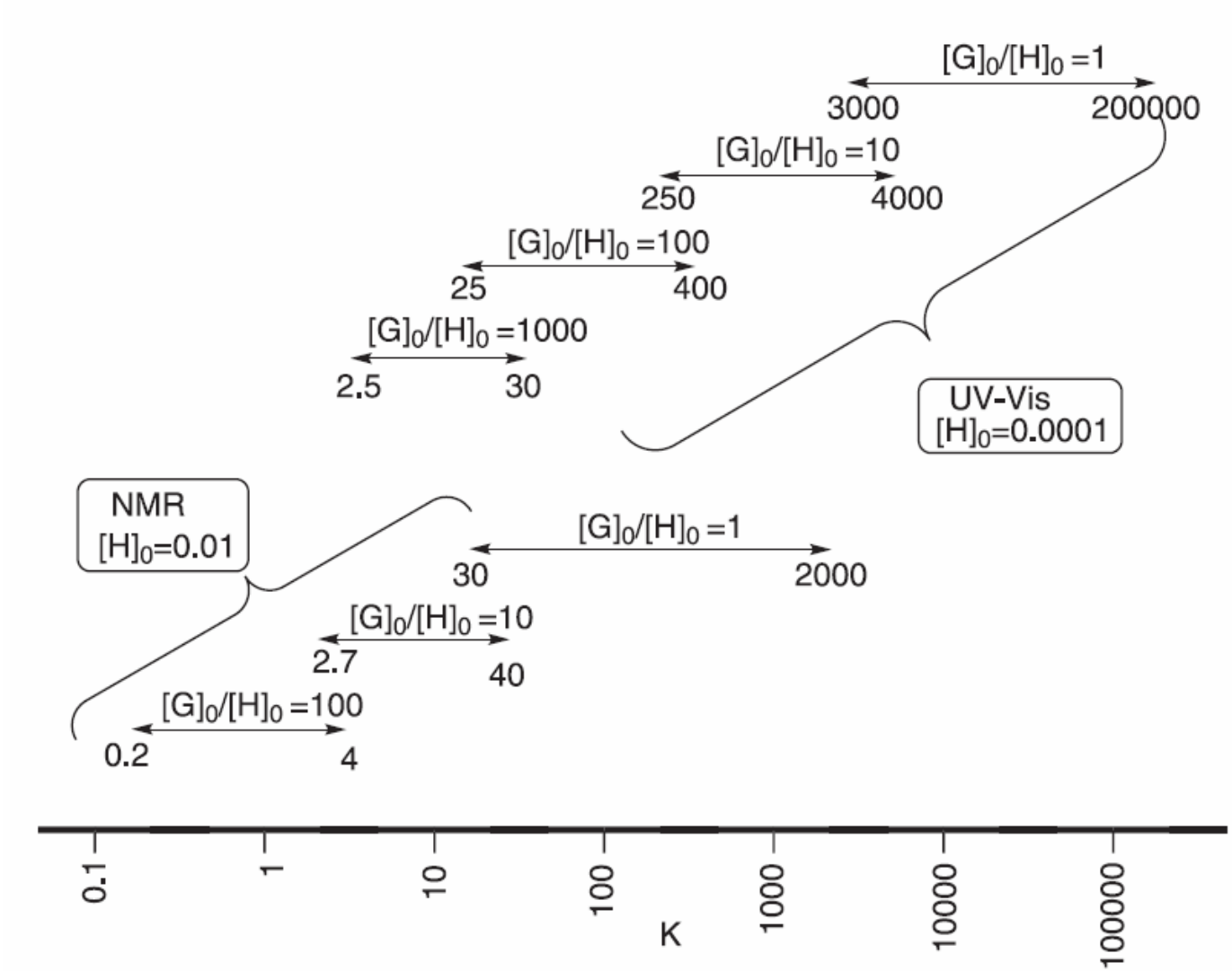


$$y = \frac{[\mathrm{C}]}{[\mathrm{H}]_0}, \quad x = \frac{[\mathrm{G}]_0}{[\mathrm{H}]_0}$$

$$\alpha = [\mathrm{H}]_0 \cdot K$$

$$[\mathrm{H}]_0 \cdot K = \frac{y}{(1-y)\cdot(x-y)}$$

$$\alpha \cdot y^2 - (\alpha + \alpha \cdot x + 1) \cdot y + \alpha \cdot x = 0$$



Reliable regions of $[H]_0$ and $[G]_0$ for *K* determination shown for representative concentrations of UV-vis and NMR experiments.

Data Treatment

- *Rose-Drago Method for UV-Vis Spectroscopy*

$$\frac{1}{K} = \frac{A_{obs} - \varepsilon_h \cdot [\mathrm{H}]_0 - \varepsilon_g \cdot [\mathrm{G}]_0}{\varepsilon_c - \varepsilon_h - \varepsilon_g} - ([\mathrm{H}]_0 + [\mathrm{G}]_0)$$

$$+ \frac{\varepsilon_c - \varepsilon_h - \varepsilon_g}{A_{obs} - \varepsilon_h \cdot [\mathrm{H}]_0 - \varepsilon_g \cdot [\mathrm{G}]_0} \cdot [\mathrm{H}]_0 \cdot [\mathrm{G}]_0$$

$$Y = \frac{1}{K}$$

$$X = \varepsilon_c - \varepsilon_h - \varepsilon_g$$

$$a_n = A_{obsn} - \varepsilon_h \cdot [\mathrm{H}]_{0n} - \varepsilon_g \cdot [\mathrm{G}]_{0n}$$

$$b_n = [\mathrm{H}]_{0n} + [\mathrm{G}]_{0n}$$

$$c_n = \frac{[\mathrm{H}]_{0n} \cdot [\mathrm{G}]_{0n}}{A_{obsn} - \varepsilon_h \cdot [\mathrm{H}]_{0n} - \varepsilon_g \cdot [\mathrm{G}]_{0n}}$$

$$Y = \frac{a_n}{X} - b_n + c_n \cdot X$$

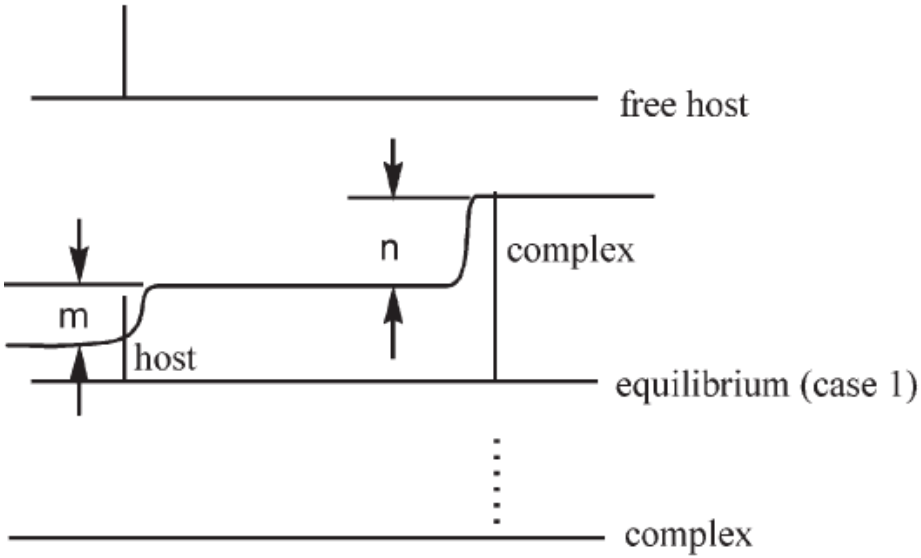
Determination of Binding Constants by NMR Spectroscopy

Case 1: The host–guest complexation equilibrium, which has a very slow exchange rate compared with the NMR time scale.

- *Determination of stoichiometry*

$$\frac{a \cdot [\mathrm{C}]}{[\mathrm{H}]_0} = \frac{n}{m+n}$$

$$\Rightarrow \frac{n}{m+n}[\mathrm{H}]_0 = a \cdot [\mathrm{C}]$$



- *Evaluation of complex concentration*

$$[\mathrm{C}] = \frac{1}{a} \cdot \frac{n}{m+n} \cdot [\mathrm{H}]_0$$

Case 2: The host–guest complexation equilibrium, which has a very fast exchange rate compared with the NMR time scale.

- *Determination of stoichiometry*

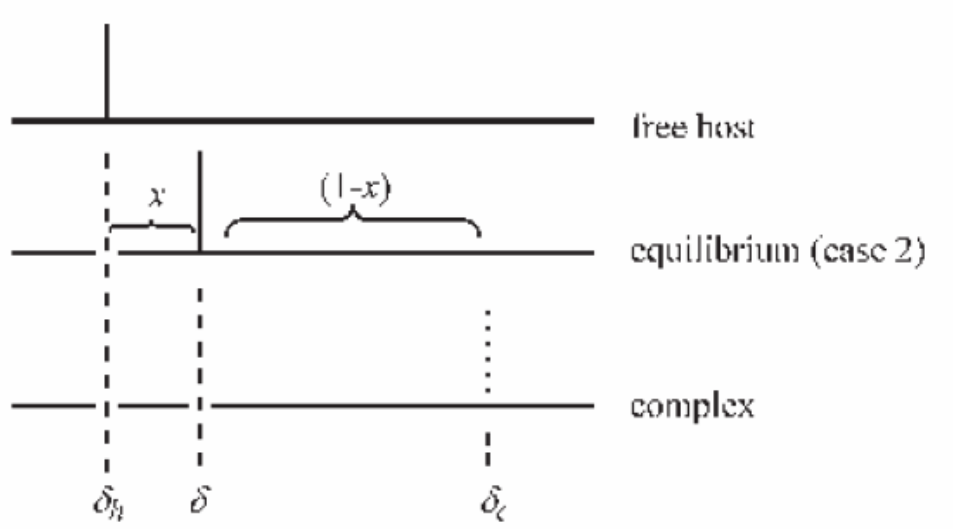
$$\delta = \delta_h \cdot (1 - x) + \delta_c \cdot x \quad \text{and} \quad x = \frac{a \cdot [\mathrm{C}]}{[\mathrm{H}]_0}$$

$$\Rightarrow [\mathrm{H}]_0 \cdot (\delta - \delta_h) = a \cdot [\mathrm{C}] \cdot (\delta_c - \delta_h)$$

δ: observed chemical shift

δ_h, δ_c: chemical shifts of the free and complexed host, respectively

x: ratio of complexed host at equilibrium over total host



- *Evaluation of complex concentration*

$$[\mathrm{C}] = \frac{1}{a} \cdot \frac{\delta - \delta_h}{\delta_c - \delta_h} \cdot [\mathrm{H}]_0$$

- *Data Treatment: Rose-Drago method (same as UV-vis)*

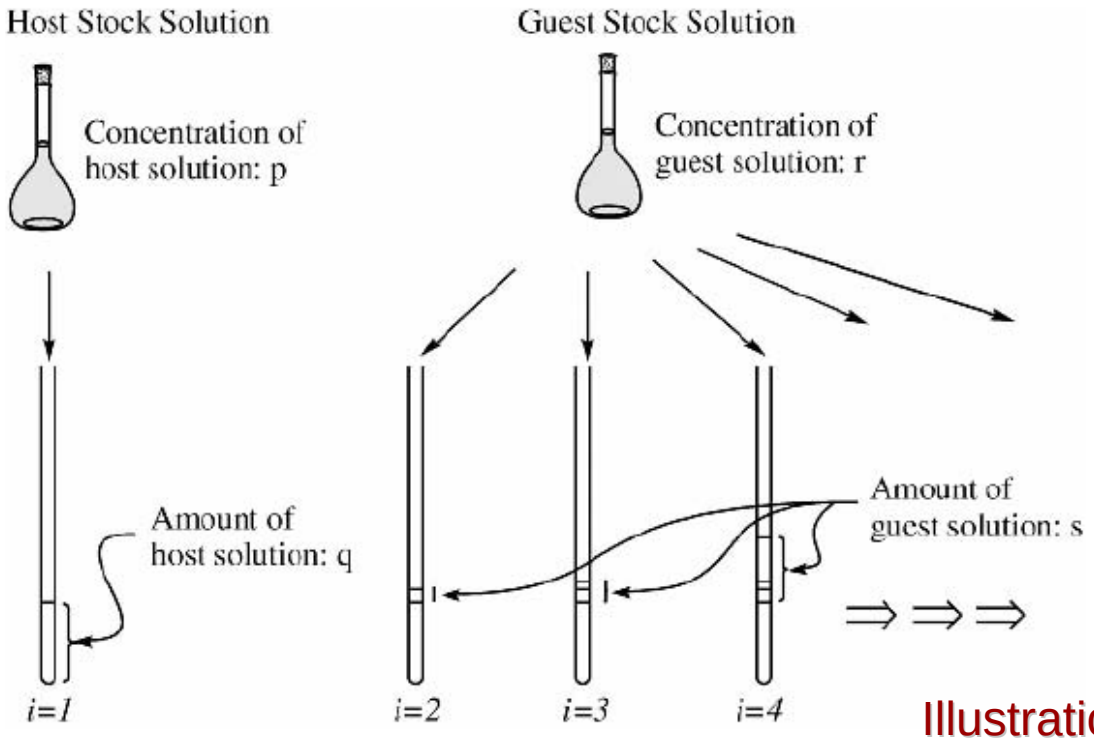


Illustration of a typical NMR titration experiment