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Review Article

A Practical Guide for the Determination of Binding Constants *

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Abstract

When working in the field of host–guest chemistry, the binding constants have to be determined on many occasions. Here is a detailed document of how to determine the binding constants which covers both the basic principle and the practical issue: a practical experimental guideline, a representative method for the determination of stoichiometry and for the evaluation of a complex concentration, precautions to be taken on setting up concentration conditions of the titration experiment, practical data-treatment methods and estimation of statistical errors. This document is described in detail using the basic level of mathematics, statistics, and programs of spreadsheet software. Especially, the titration experiments by means of UV-visible and NMR spectroscopy are carried out and described.

Introduction

In order to appreciate the extent of an intermolecular binding between a host and a guest, this article describes two impressive examples performed by our research group. Both are realized based on the different extent of complex formation of a chiral host with a chiral guest. In Figure 1, chiral recognition by a brilliant color change using chromophoric chiral crown ether **1** upon complexation with the *R* and *S* enantiomers of phenylglycinol **2** is shown. The solution color of chiral crown ether host **1** in chloroform at 25 °C is yellow (Figure 1a). When (*R*)-phenylglycinol ((*R*)-**2**) is added to the solution, the color changes from yellow to purple (Figure 1b). In contrast, when (*S*)-**2** is added to the solution, the color remains yellow (Figure 1c). Because of the high enantioselectivity of host **1** toward amine **2**, the chirality of **2** can thus be readily identified from the color of the solution by the naked eye. This color change is related to the complex formation in this example [1] The other example is a facile FAB mass spectrometric approach for the chiral recognition in host-guest complexation determined by the enantiomer-labeled guest method, which is shown in Figure 2. The FAB mass spectra of host **3** with a 1 : 1 mixture of (*S*)-methionine methyl ester (*S*)-**4** and (*R*)-methionine methyl ester-d_3 ((*R*)-**4**-d_3) are shown in Figure 2. The mass peak at mass number 780 corresponds to the complex of **3** with (*S*)-**4**, and that at 783 corresponds to that with (*R*)-**4**-d_3. The different peak intensities between these diastereomeric pairs of the complexes, (**3**+(*S*)-**4**) and (**3**+(*R*)-**4**-d_3), are related to the intermolecular binding between each host and guest in this example. From the basic chemical interest, the extent of the enantioselectivity is required to be shown using general parameters such as the binding constant (K), enthalpy change (ΔH), entropy change (ΔS), and free energy (ΔG) of complex formation instead of a brilliant color change or an appreciable difference in peak intensities.

Generally speaking, the formation of a complex between host and guest is a basic and important process in supramolecular chemistry. The binding constant has to be determined for the quantitative analysis [3–5] of the complex formation. In spite of the importance of determining the binding constant, it is still difficult to find documentation, where the practical issues are mentioned. Some previous documents include the practical issue, but which is covered over a large area with the explanation of basic data-treatment methods. The introduction of many different types of approximation and regression methods was important at that time from the practical point of view, in order to find an appropriate method for a wide range of certain experiments, because each approximation has severe limitation in application. Such methods do not meet the needs of a chemist nowadays. The situation is considerably improved by data-treatment through computer development. A personal computer is easily accessible and time-saving and also provides accurate results. On the other hand, recently there are a few review articles [6–8] which are frequently cited in papers describing the basic principle to determine the binding constants.

This document describes in detail how to determine the binding constants, covering not only the basic principle but also the practical issue: a theoretical experimental guideline, a representative method for the determination of

* **Supplementary Data** relating to this article are deposited with the British Library as Supplementary Publication No. 82279 (4 pp).

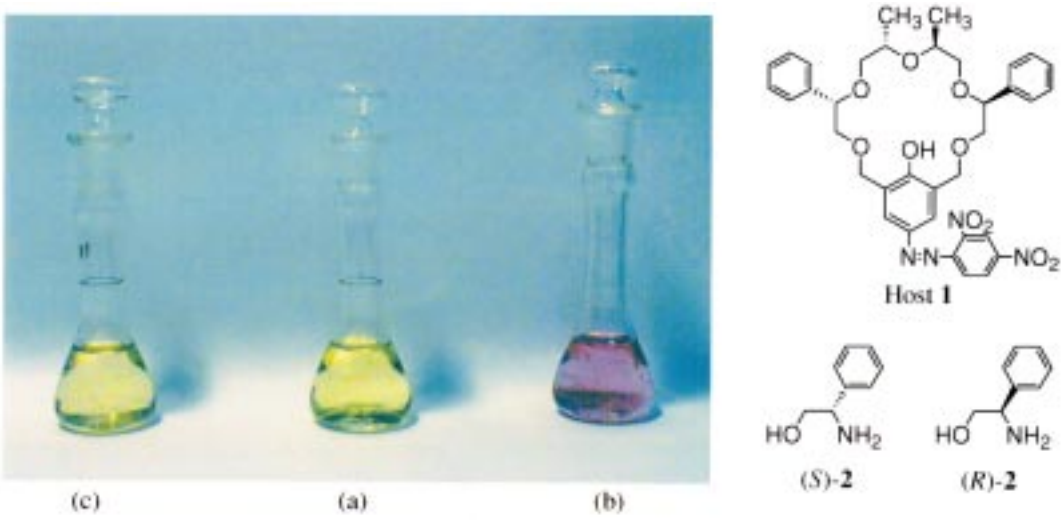


Figure 1. (a) A chloroform solution of (*S*, *S*, *S*, *S*)-**1** (1×10^{-5} M) at 25 °C; (b) The same solution as (a) containing 1 equivalent of (*R*)-**2**; (c) The same solution as (a) containing 1 equivalent of (*S*)-**2**.

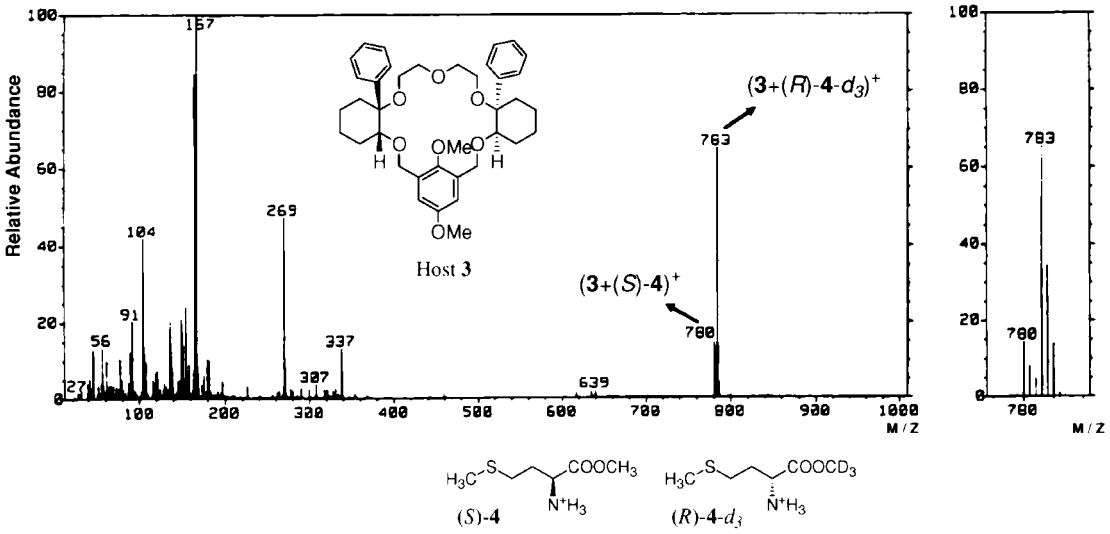


Figure 2. FAB mass spectrum for the complexation between host **3** and guest **4** (1 : 1 mixture of (*S*)-**4** and (*R*)=**4**-d_3).

stoichiometry and for the evaluation of complex concentration, precautions to be taken on setting up concentration conditions of the titration experiment, practical data-treatment methods and estimation of the statistical errors for those who are doing host-guest chemistry but are not yet very familiar with this kind of work, mostly synthesis-oriented organic chemists. The programs for determination of binding constants of host-guest complexation were developed using the spreadsheet software on a personal computer. The simplified programs are attached to this document as Appendices.

Theory

General view to determine the binding constants

Comprehension and interpretation of basic equations for host–guest complexation

The binding constant is used as a criterion for the evaluation of the host-guest complexation process. Thermodynamic parameters (enthalpy, entropy) and Gibbs free energy are more suitable criteria. In the case where Equations (1) and (2) hold good, thermodynamic parameters are related to each other as described in Figure 3 and Equation (3), the van't Hoff equation. Theoretically, the determination of binding constants at different temperatures offers these thermodynamic parameters from the slope and intercept of the line in Figure 3. *The important point in the quantitative analysis of host-guest complexation is how to determine the binding constant with high reliability*.

$$\Delta G = -RT \ln K \tag{1}$$

$$\Delta G = \Delta H - T\Delta S \tag{2}$$

$$\therefore \ln K = -\frac{\Delta H}{R} \cdot \frac{1}{T} + \frac{\Delta S}{R}. \tag{3}$$

Our analysis to determine the binding constant is based on a simple binding equilibrium model: Equation (4). The bind-

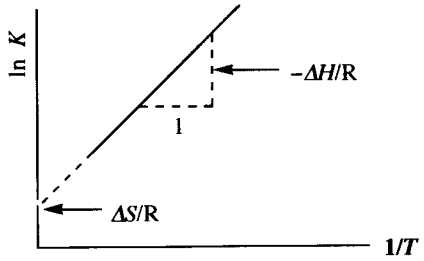


Figure 3. Correlation of thermodynamic parameters, K and temperature according to van't Hoff equation.

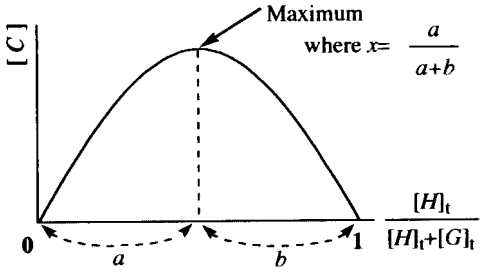


Figure 4. Correlation between stoichiometry (a, b) and x-coordinate at the maximum of the curve in Job's plot.

ing constant, equilibrium constant, and stability constant are synonymous with each other. The activity coefficients are generally unknown and the stability constant K, based on the concentrations, is usually employed. Judging from this situation, the question of the activity coefficients of the solutes is disregarded here in order to simplify the discussion. Nevertheless, it should be remembered that this point is not always insignificant. The basic equations for host–guest complexation are the following four Equations (4)–(7).

$$a \cdot H + b \cdot G \rightleftharpoons C \tag{4}$$

$$K = \frac{[C]}{[H]^a \cdot [G]^b} \tag{5}$$

$$[H]_t = [H] + a \cdot [C] \tag{6}$$

$$[G]_t = [G] + b \cdot [C], \tag{7}$$

where H is host; G, guest; C, complex: $H_a \cdot G_b$; a, b, stoichiometry: shown in Equation (4); $[H]_t$, total concentration of host molecule at initial state; $[G]_t$, total concentration of guest molecular at initial stage; $[H]$, $[G]$, $[C]$, concentrations of host, guest, and complex respectively at final stage, namely, at equilibrium. Equation (8) is derived from Equations (5)–(7).

$$K = \frac{[C]}{([H]_t - a \cdot [C])^a \cdot ([G]_t - b \cdot [C])^b}. \tag{8}$$

Parameters are classified into three as follows. Constants: K, a, b (a and b are integers larger than or equal to 1). Variables which can be set up as experimental condition: $[H]_t$, $[G]_t$. Variables dependent on each equilibrium: $[H]$, $[G]$, $[C]$.

Experimental guideline from the theory

From Equation (8) and the classification of its parameters is elucidated the guideline of the experiment. When $[C]$ is obtained under the equilibrium in which a and b are known, K is derived directly according to Equation (8) from the experimental condition $[H]_t$, and $[G]_t$. Consequently, in order to determine the binding constants, the following four tasks have to be carried out.

- Determination of stoichiometry, namely, a and b
- Evaluation of $[C]$
- Setting up the concentration conditions $[H]_t$ and $[G]_t$
- Data-treatment

The following sections deal with the principle and also the practical issues necessary for an understanding and completion of the above four tasks in this order.

Experiment (practical measurement)

Determination of stoichiometry

Continuous variation methods

There are different methods of determining the stoichiometry, e.g., Continuous Variation Methods [10], Slope Ratio Method [11], Mole Ratio Method [12], etc. Because the Continuous Variation Method is the most popular among these, this method is adopted here to determine the stoichiometry.

In order to determine the stoichiometry by the Continuous Variation Method, the following four points have to be considered and carried out.

- Keeping the sum of $[H]_t$ and $[G]_t$ constant (α)
- Changing $[H]_t$ from 0 to α
- Measuring $[C]$
- Data treatment (Job's plot)

The stoichiometry $(a/(a + b))$ is obtained from the x-coordinate at the maximum in Job's curve (Figure 4), where the y-axis is $[C]$ and the x-axis is.

$$\frac{[H]_t}{([H]_t + [G]_t)}.$$

For the comprehension of the theoretical background of the Continuous Variation Method, the required Equations are (4)–(7) and (9)–(11).

$$\alpha = [H]_t + [G]_t \tag{9}$$

$$x = \frac{[H]_t}{([H]_t + [G]_t)} \tag{10}$$

$$y = [C]. \tag{11}$$

$[H]_t$ and $[G]_t$ will be substituted by the function of x and α from Equations (9) and (10).

$$[H]_t = \alpha \cdot x \tag{12}$$

$$[G]_t = \alpha - \alpha \cdot x, \tag{13}$$

from Equations (4)–(7) and (11)–(13).

$$K = \frac{y}{\{(\alpha - b \cdot y - \alpha \cdot x)^b \cdot (\alpha \cdot x - a \cdot y)^a\}}$$

$$K \cdot (\alpha - b \cdot y - \alpha \cdot x)^b \cdot (\alpha \cdot x - a \cdot y)^a = y. \qquad (14)$$

Equation (14) is then differentiated, and the $\mathrm{d}y/\mathrm{d}x$ is substituted by zero. Then the x-coordinate at the maximum in the curve is obtained.

$$\begin{aligned} K \cdot & \left[(\alpha - b \cdot y - \alpha \cdot x)^b \cdot \{(\alpha \cdot x - a \cdot y)^a\}'\right. \\ & \left. + \{(\alpha - b \cdot y - \alpha \cdot x)^b\}' \cdot (\alpha \cdot x - a \cdot y)^a\right] = \frac{\mathrm{d}y}{\mathrm{d}x} \\ K \cdot & \left[(\alpha - b \cdot y - \alpha \cdot x)^b \cdot a \cdot (\alpha \cdot x - a \cdot y)^{a-1}\right. \\ & \cdot \left(\alpha - a \cdot \frac{\mathrm{d}y}{\mathrm{d}x}\right) + b \cdot (\alpha - b \cdot y - \alpha \cdot x)^{b-1} \\ & \left. \cdot \left(-b \cdot \frac{\mathrm{d}y}{\mathrm{d}x} - \alpha\right) \cdot (\alpha \cdot x - a \cdot y)^a\right] = \frac{\mathrm{d}y}{\mathrm{d}x}. \end{aligned}$$

The substitution of $\mathrm{d}y/\mathrm{d}x$ by zero is derived as follows.

$$\begin{aligned} K \cdot & [(\alpha - b \cdot y - \alpha \cdot x)^b \cdot a \cdot (\alpha \cdot x - a \cdot y)^{a-1} \cdot \alpha \\ & + b \cdot (\alpha - b \cdot y - \alpha \cdot x)^{b-1} \\ & \cdot (-\alpha) \cdot (\alpha \cdot x - a \cdot y)^a] = 0. \end{aligned}$$

Subtraction by $K \cdot (\alpha - b \cdot y - \alpha \cdot x)^{b-1} \cdot (\alpha \cdot x - a \cdot y)^{a-1} \cdot \alpha$ produces

$$a \cdot (\alpha - b \cdot y - \alpha \cdot x) - b \cdot (\alpha \cdot x - a \cdot y) = 0$$

$$a \cdot \alpha - a \cdot b \cdot y - a \cdot \alpha \cdot x - b \cdot \alpha \cdot x + b \cdot a \cdot y = 0$$

$$a \cdot \alpha - a \cdot \alpha \cdot x - b \cdot \alpha \cdot x = 0.$$

Subtraction by α.

$$a - ax - bx = 0$$

$$\therefore x = \frac{a}{a+b}. \qquad (15)$$

Equation (15) means $a/a+b$ is the x-coordinate at the maximum ($\mathrm{d}y/\mathrm{d}x = 0$) in the curve of Equation (14). Equation (15) shows the correlation between the stoichiometry and the x-coordinate at the maximum in Job's plot. For example, when 1 : 1 complexation is predominant at equilibrium, the maximum appears $x = 0.5$ ($a = b = 1$). In the case of 1 : 2 complexation $x = 0.333$ gives the maximum.

The practically important point here is the following. *Even if the concentration of the complex ([C]) could not be measured directly, the [C] (y-axis) would be replaced with a property proportional to [C]. Then the same x-coordinate can be obtained at the maximum as that in Job's plot.* This means the stoichiometry can be determined even if $[C]$ could not be obtained. The important point is how to modify the y-coordinate.

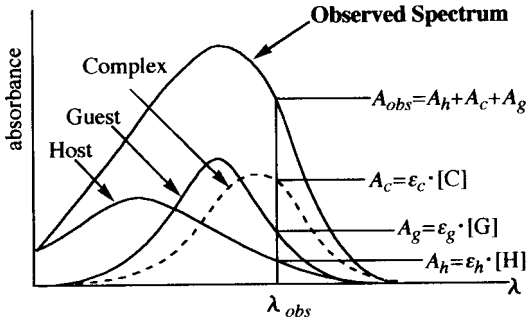


Figure 5. Representative UV-visible spectra to show correlation of observed spectra and each component.

Depending on each experiment, there is a property which is suitable for the replacement of $[C]$. Concerning the UV-visible and NMR spectroscopies, three examples are mentioned below.

• UV-visible Spectroscopy

In the case of investigation by means of UV-visible spectroscopy, the concentrations and absorbances of each species are related by the following equations (16)–(18). And the observed absorbance is expressed as Equation (19) and Figure 5. The length of the cell is fixed here to 1 cm as a premise. The definitions of the abbreviations are given below. The definitions of other abbreviations (a, b, $[H]_t$, $[G]_t$, $[H]$, $[G]$, $[C]$) are the same as described before (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).

$$A_h = \epsilon_h \cdot [H] = \epsilon_h \cdot ([H]_t - a \cdot [C]) \qquad (16)$$

$$A_g = \epsilon_g \cdot [G] = \epsilon_g \cdot ([G]_t - b \cdot [C]) \qquad (17)$$

$$A_c = \epsilon_c \cdot [C] \qquad (18)$$

$$A_{\mathrm{obs}} = A_h + A_g + A_c. \qquad (19)$$

Equation (19) is transformed to Equation (20) by using Equations (16)–(18).

$$\begin{aligned} A_{\mathrm{obs}} &= \epsilon_h \cdot ([H]_t - a \cdot [C]) + \epsilon_g \cdot ([G]_t - b \cdot [C]) \\ &\quad + \epsilon_c \cdot [C] \\ &\quad \therefore A_{\mathrm{obs}} - \epsilon_h \cdot [H]_t - \epsilon_g \cdot [G]_t \\ &= (\epsilon_c - a \cdot \epsilon_h - b \cdot \epsilon_g) \cdot [C]. \end{aligned} \qquad (20)$$

Equation (20) shows that $A_{\mathrm{obs}} - \epsilon_h \cdot [H]_t - \epsilon_g \cdot [G]_t$ is proportional to $[C]$ because $(\epsilon_c - a \cdot \epsilon_h - b \cdot \epsilon_g)$ is constant. The molar absorptivities ϵ_h, ϵ_g are determined from other measurements using the pure host and pure guest, respectively. The concentrations $[H]_t$ and $[G]_t$, are known because they are experimental conditions. Consequently, $(A_{\mathrm{obs}} - \epsilon_h \cdot [H]_t - \epsilon_g \cdot [G]_t)$ is determined from the experiments

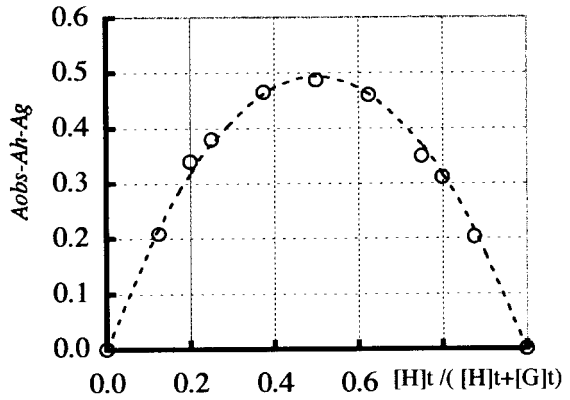


Figure 6. Modified Job's plot for complexation of host and guest by UV-visible spectroscopy. ○: observed; ——: calculated.

by means of UV-visible spectroscopy. The stoichiometry is determined from the x-coordinate at the maximum in the curve which might be called a modified Job's plot where $(A_{\text{obs}} - \epsilon_h \cdot [H]_t - \epsilon_g \cdot [G]_t)$ is plotted as the y-coordinate instead of $[C]$.

An actual example of the modified Job's plot is shown in Figure 6. The x-coordinate at the maximum in the curve is 0.5. This supports the 1 : 1 host-guest complexation. For a better feeling of the practical experiment, a spreadsheet for the Continuous Variation Method is attached as Appendix 1.

• NMR Spectroscopy

Concerning the NMR spectrometric method, it should be classified into two cases by the difference in the exchange rate. In the case where the host-guest complexation equilibrium has a similar exchange rate compared to the NMR time scale, the NMR peaks broaden and/or disappear, so it is impossible to measure. There are the following two cases suitable for the measurement by NMR spectroscopy.

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

In this case the peaks which are assigned to the host parts in the complex and those to the free host are observed individually in the same NMR spectrum. Those peaks appear at individual chemical shifts. In Figure 7, there is a representative NMR spectrum where the peaks, which are assigned to a free or complexed host, are observed individually with the integration ratio m to n. The composition of the complex is H_aG_b. Then, the integration of the host parts in the complex over the total integration of the host parts is as follows.

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

$$\therefore \frac{n}{m+n}[H]_t = a \cdot [C]. \tag{21}$$

The stoichiometry is determined from the x-coordinate at the maximum in the curve which might be called a modified Job's plot where $(n/(m+n)) \cdot [H]_t$ is plotted as the y-coordinate instead of $[C]$, for the following reasons.

- Equation (21) means that $(n/(m+n)) \cdot [H]_t$ is proportional to $[C]$ since a is constant.
- $[H]_t$ can be set up in the experimental condition.
- The ratio of $n/(m+n)$ is obtained from the NMR spectral data.

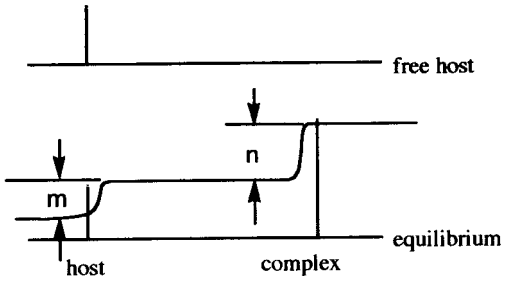


Figure 7. Representative NMR spectra for slow exchange of the complexation equilibrium.

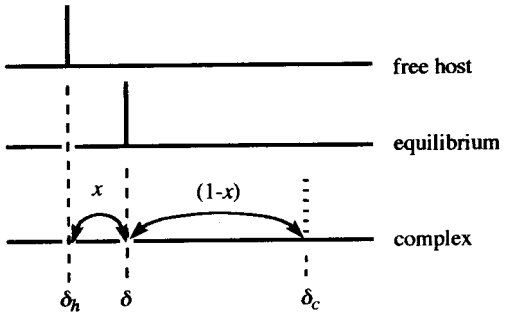


Figure 8. Representative NMR spectra for fast exchange of complexation indicating a correlation of the complexation ratio x and each spectrum.

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

In this case the peaks which are assigned to the host parts in the complex and those to the free host are fused. In Figure 8, there is a representative NMR spectrum where the peaks, which are assigned to the free and complexed host parts, are fused and appear at the weight average chemical shift of the free host and complexed host. In this case: δ, observed chemical shift; δ_h, δ_c, chemical shifts of the host part in free and complexed host, respectively; x, ratio of complexed host at equilibrium over total host

$$\delta = \delta_h \cdot (1-x) + \delta_c \cdot x \text{ where } x = \frac{a \cdot [C]}{[H]_t}$$

$$\therefore [H]_t \cdot (\delta - \delta_h) = a \cdot [C] \cdot (\delta_c - \delta_h). \tag{22}$$

The stoichiometry is determined from the x-coordinate at the maximum in the curve which might be called a modified Job's plot where $[H]_t \cdot (\delta - \delta_h)$ is plotted as y-coordinate instead of $[C]$ for the following reasons.

- Equation (22) means that $[H]_t \cdot (\delta - \delta_h)$ is proportional to $[C]$, since $a \cdot (\delta_c - \delta_h)$ is constant.
- $[H]_t$ can be set up in the experimental condition.
- The $(\delta - \delta_h)$ are obtained from the NMR spectral data.

Evaluation of complex concentration

Case study

When the observed property is the complex concentration ($[C]$) at equilibrium itself, there is no difficulty. But the actual complex concentration is not observed directly in most cases. How to evaluate $[C]$ is an important point. The practical way depends on the property that can be observed in each experiment. In this section, representative examples for the evaluation of complex concentration at equilibrium are given: two for the UV-visible spectrometric method and two for the NMR spectrometric method.

(a) UV-visible Spectroscopy

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

From Equation (20), the following equation (23) is derived.

$$[C] = \frac{A_{\text{obs}} - \epsilon_h \cdot [H]_t - \epsilon_g \cdot [G]_t}{\epsilon_c - a \cdot \epsilon_h - b \cdot \epsilon_g}. \tag{23}$$

If all the constants ($a, b, \epsilon_h, \epsilon_g$ and ϵ_c) were known, $[C]$ would be determined using the experimental condition ($[H]_t, [G]_t$) and the observed property (A_{obs}). Since the molar absorptivity of the complex (ϵ_c) is not measurable directly, a titration experiment and regression are necessary for the evaluation of the complex concentration. This is the most complicated case of host-guest complexation detecting by means of UV-visible spectroscopy because the absorption bands of all components, the host, guest and complex, are overlapped. When one component (e.g., the guest) whose ϵ is zero, is used, the following simplification is applied. Even if ϵ is not zero, the simplification would be carried out normally in such a way that the detection-wavelength is adjusted so that the absorption band of one component (e.g., the guest) is not overlapped with those of other species (e.g., the host and complex).

- Case 2: the absorption bands of two components are overlapped

The equation for this case is expressed by Equation (24), which is derived just by substitution of ϵ_g by zero from Equation (23).

$$[C] = \frac{A_{\text{obs}} - \epsilon_h \cdot [H]_t}{\epsilon_c - a\epsilon_c - a \cdot \epsilon_h}. \tag{24}$$

Compared to Equation (23), Equation (24) is simplified. Because three parameters (b, ϵ_g, and $[G]_t$) disappear from Equation (23), data-treatment is much simplified. If all constants (a, ϵ_h and ϵ_c) were obtained, $[C]$ would be determined using the experimental condition ($[H]_t$). *Since the molar absorptivity of complex (ϵ_c) is not measurable directly, a titration experiment and regression are necessary for the evaluation of the complex concentration in this case.*

(b) NMR Spectroscopy

The following two examples are representative cases for the experiment by means of NMR spectroscopy.

- Case 1: The host-guest complexation equilibrium has a very slow exchange rate compared to the NMR time scale

Equation (25) is derived from Equation (22) just by simple transformation. When the stoichiometry (a) is obtained, $[C]$ is determined using the experimental condition ($[H]_t$) according to Equation (25), Since $n/m + n$ is obtained from the NMR measurement.

$$[C] = \frac{1}{a} \cdot \frac{n}{m+n} \cdot [H]_t. \tag{25}$$

- Case 2: The host-guest complexation equilibrium has a very fast exchange rate compared to the NMR time scale

This case is often observed for complexation with a crown ether and an amine. Equation (26) is derived from Equation (22) just by simple transformation.

$$[C] = \frac{1}{a} \cdot \frac{\delta - \delta_h}{\delta_c - \delta_h} \cdot [H]_t. \tag{26}$$

If all constants (a, δ_h, δ_g and δ_c) were obtained, $[C]$ would be determined using the experimental condition ($[H]_t$). Since δ_c is not obtained directly, a titration experiment and regression are necessary for the evaluation of the complex concentration.

The background of the principle has now been provided in the above sections concerning the kind of experiment for the following points.

- How to determine the stoichiometry: Continuous Variation Method
- How to evaluate the concentration of complex: Titration Experiment and Regression

Precautions to be taken on setting up the concentration conditions of the titration experiment

- Correlation between $[H]_t$, $[G]_t$, x and K

Each method for binding analysis has limitations. There are dangerous sources of systematic error that are often encountered in host-guest complexation, that is, the danger of carrying out titrations at concentrations unsuitable for the equilibrium being measured. The origin of this error will be discussed and methods for avoiding these problems will be presented in the following two sections.

The experimental conditions that can be set up are $[H]_t$ and $[G]_t$ (see Equation (8) and classification of variables). How should the experimental conditions, $[H]_t$ and $[G]_t$, be changed for the titration? There are many possibilities described in Figure 9. The criteria to decide the way of change might be as follows.

- Reliability (Small Error)
- Easy for experiment and calculation
- Applicability
- Acceptability

Here we think about the way to set up the experimental condition, $[H]_t$ and $[G]_t$ using the host-guest 1 : 1 complexation.

$$H + G \rightleftharpoons C. \tag{27}$$

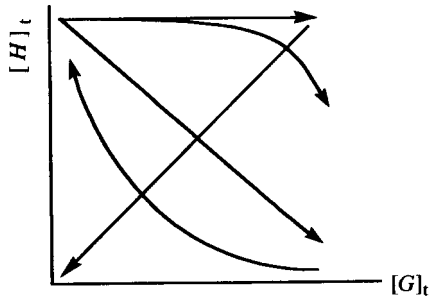


Figure 9. Graphical expression showing possible ways to change $[H]_t$ and $[G]_t$ for a titration experiment.

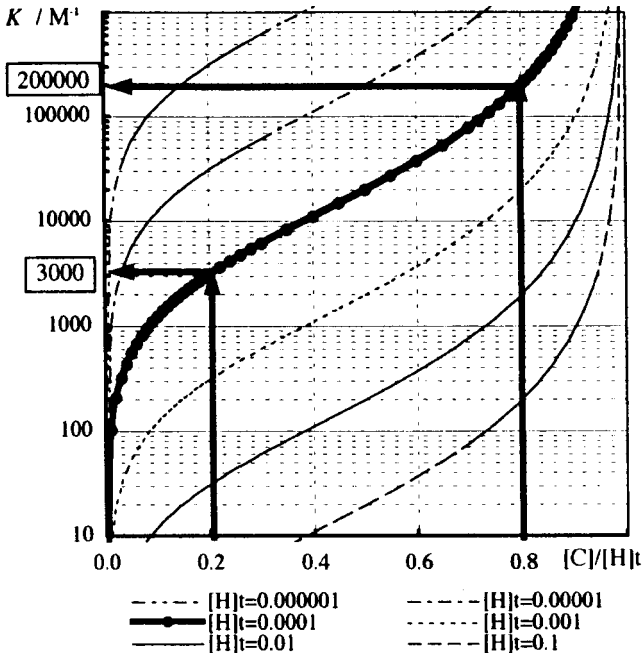


Figure 10. The correlation between the complexation ratio (x) and the binding constant (K).

From Equation (8)

$$K = \frac{[C]}{([H]_t - [C]) \cdot ([G]_t - [C])}. \tag{28}$$

Let us figure the correlation between K and the complexation ratio (x).

$$y = K, x = \frac{[C]}{[H]_t}, \tag{29}$$

then Equation (28) is transformed to

$$y = \frac{x}{(1 - x) \cdot ([G]_t - [H]_t \cdot x)}. \tag{30}$$

Figure 10 is the graph of Equation (30) where x (x-coordinate) is 0 to 1, K (y-coordinate) is 10 to 1,000,000, and $[H_t]$ is 0.1 to 0.000001, $[H]_t = [G]_t$ as a premise. In general, caution is expressed as: "measurements below 20% and above 80% complexation ratio (x) yield uncertain values". This caution is interpreted with Figure 10 as follows.

The steep rises of K in the complexation ratio ranges less than 20% and more than 80% cause transfer of error from

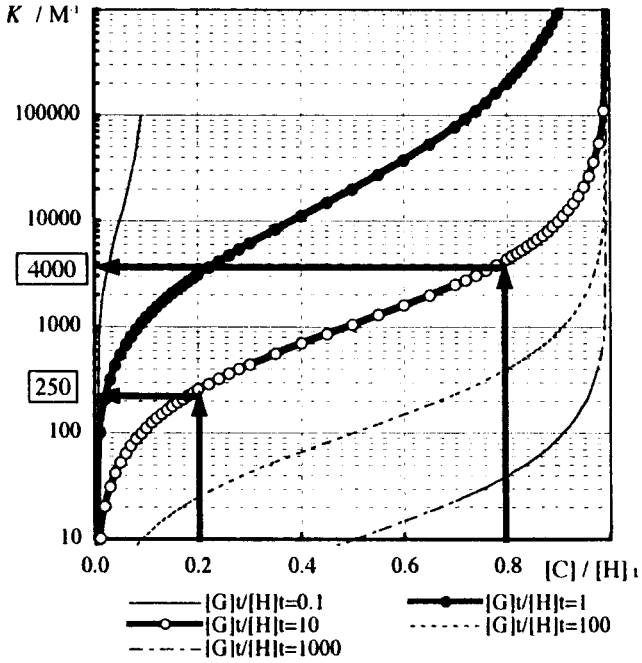


Figure 11. The correlation between the complexation ratio (x) and the binding constant (K).

the complexation ratio into K with magnification. When K is determined based on the measurement of the property directly connected to or proportional to the complexation ratio, the obtained K value will have magnified errors. In the case where $[H]_t$ = 0.0001 M is an example, the complexation ratio becomes 0.2–0.8 when K is between 3000 to 200,000 M^{-1}. So an accurate experiment is carried out. In this way the accuracy of K is governed by the setting up of concentrations, $[H]_t$, $[G]_t$ and also K itself.

Since setting up the concentration of host $[H]_t$ is limited by the measuring properties, apparatus, etc., of the experiment, $[G]_t$ is the only variable to be set up in a wide range. For example, $[H]_t$ for NMR spectroscopy is roughly in the range of 0.01 mol/l with one or two order variations. $[H]_t$ for UV-visible spectroscopy, which depends severely on the molar absorptivity, is roughly in the range of 0.0001 mol/l. Then what is the best way to set up the concentration of $[G]_t$? In order to consider this problem, Figure 11 is drawn based on Equation (30) where $[H]_t$ = 0.0001 and $[G]_t/[H]_t$ is changed from 0.1 to 1000 as shown in Figure 11.

The correlation between the complexation ratio x and the accurately obtainable K range by changing $[G]_t$ with constant $[H]_t$ (= 0.0001 mol/l) is clear based on Figure 11. Considering the suitable x range ($0.2 < x < 0.8$) for reliable measurement in Figure 11, the combination of $[H]_t$, $[G]_t$ and K is determined. For example, when $[G]_t$ = 0.001 mol/l, and $[H]_t$ = 0.0001 mol/l, then $[G]_t/[H]_t = 10$, consequently, a reliable range of K (250-4000 M^{-1}) is obtained by following the arrows in Figure 11.

By repeating these procedures for several combinations of $[H]_t$, $[G]_t$, the obtained K ranges are summarized in Figure 12. This Figure is useful for a preliminary check of the experimental concentration condition.

- How to set up $[G]_t$

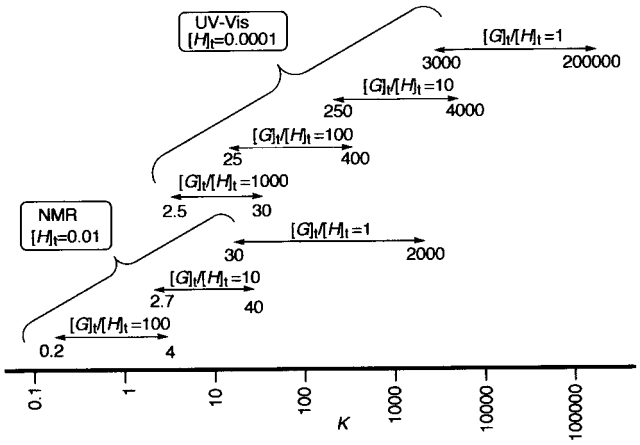


Figure 12. Reliable regions of $[H]_t$ and $[G]_t$ for K determination shown for representative construction of UV-visible and NMR spectroscopies.

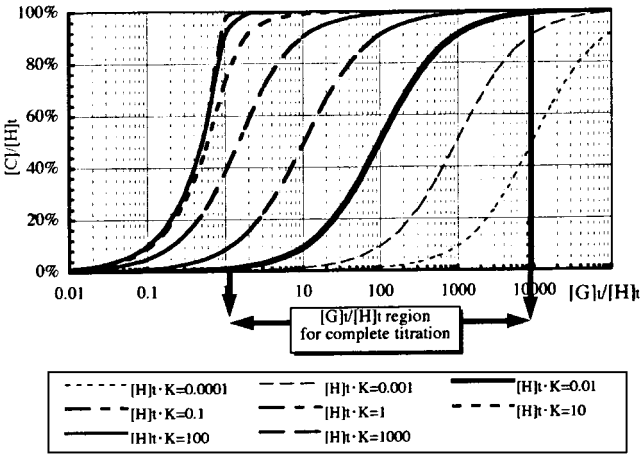


Figure 13. The calculated curves plotted between $[G]_t/[H]_t$ and $[C]/[H]_t$ – useful graph for $[G]_t$ range determination of the titration experiment.

The same problem is discussed here according to the experiment. In most cases, K is determined based on the titration experiment followed by regression of the obtained data with a theoretical equation. As regards the normal titration experiment, $[G]_t$ is changed under the condition where the range of $[H]_t$ change is limited. In such a case, the important point is how to set up the range of $[G]_t$. The following is one representative answer.

First of all, the correlation between $[G]_t/[H]_t$ and the complexation ratio is considered based on Figure 13 where the x-coordinate is the concentration ratio of the guest over host mixed in the cell and the y-coordinate is the complexation ratio. The graph in Figure 13 is based on Equation (33) which is derived from Equation (28) by multiplying both sides of the equation by $[H]_t$, dividing the denominator and numerator by $[H]_t^2$, then substituting with y and x according to Equation (31).

$$y = \frac{[C]}{[H]_t}, x = \frac{[G]_t}{[H]_t} \tag{31}$$

$$[H]_t \cdot K = \frac{y}{(1-y)\cdot(x-y)}. \tag{32}$$

Displacement using the equation $\alpha = [H]_t \cdot K$ and transformation produces

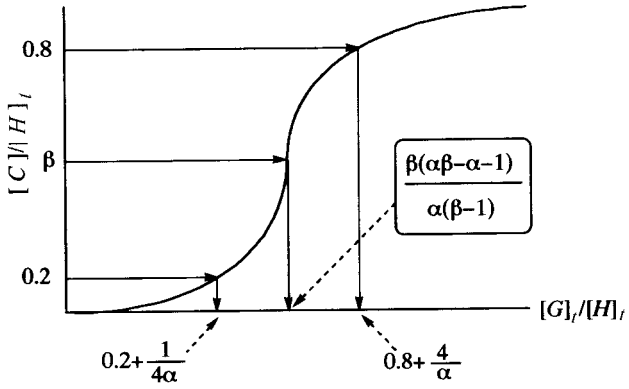


Figure 14. Useful graph for $[G]_t$ range determination of titration experiment ($\alpha = [H]_t \cdot K$, $\beta = [C]/[H]_t$).

$$\alpha \cdot y^2 - (\alpha + \alpha \cdot x + 1)\cdot y + \alpha \cdot x = 0. \tag{33}$$

Figure 13 is obtained by changing α from 0.00001 to 1000, which corresponds to the change of K from $K = 0.00001/[H]_t$ to $K = 1000/[H]_t$. Though tracing from the bottom to the top of the S-curve in Figure 13 should be necessary for complete identification of each equilibrium, it is possible to determine the binding constant by plotting the data $[C]/[H]_t$, $[G]_t/[H]_t$ as Figure 13 which are obtained from experiment, followed by curve-fitting using Equation (33). When $[H]_t \cdot K = 0.01$ is picked up as an example, the range of $[G]_t$ for complete titration is $1 \cdot [H]_t$–$10000 \cdot [H]_t$ mol/l as indicated in Figure 13. In order to reduce error, the $[G]_t$ area where lines are close together should be avoided. When the experimental condition is in a crowded area, a small error in $[G]_t$ causes a plot on a different S-curve whose K is much different. Then this unsuitable concentration setting results in the low reliability of the calculated K value. From this consideration, the range of the complexation ratio between 0.2 to 0.8 is suitable here again for reliable measurement. The suitable range of $[G]_t$ could be obtained from Figure 13. For this example, the suitable range of $[G]_t$ is $25 \cdot [H]_t$–$400 \cdot [H]_t$. This is the range expressed relative to $[H]_t$. In order to obtain this suitable range of $[G]_t$, Figure 14 is drawn as follows.

From Equation (33) it is possible to express the x-coordinate using α and β as follows,

$$x = \frac{\beta \cdot (\alpha \cdot \beta - \alpha - 1)}{\alpha \cdot (\beta - 1)} \tag{34}$$

$$x = \frac{[G]_t}{[H]_t}, \alpha = [H]_t \cdot K, \beta = \frac{[C]}{[H]_t}.$$

The complexation ratio here is β. With Equation (34) the $[G]_t$ range for the titration experiment where the complexation ratio 0.2–0.8 is obtained as functions of α just by inputting β = 0.2 or β = 0.8 into Equation (34). The result is summarized in Figure 14. On inputting α = 0.01, then the suitable x range is easily obtained from Figure 14.

$$25.2 = 0.2 + \frac{1}{4 \cdot 0.01} \leq \frac{[G]_t}{[H]_t} \leq 0.8 + \frac{4}{0.01} = 400.8.$$

One more consideration for the $[G]_t$ setting is mentioned here. Look at Figure 13 again. When $[H]_t \cdot K$ is larger than one, the curves are close together even if the complexation ratio is between 0.2 and 0.8. Consequently, as a premise for reliable experiment, $[H]_t \cdot K$ should be *smaller than one*. When $[H]_t \cdot K$ is larger than one, $[H]_t$ should be reduced. When $[H]_t$ cannot be reduced, the observed physical property and the spectroscopy should be changed.

From Figure 14, the $[G]_t$ range is limited as described below.

$$0.2+\frac{1}{4\cdot\alpha} \le \frac{[G]_t}{[H]_t} \le 0.8+\frac{4}{\alpha} \quad \text{where } \alpha = [H]_t \cdot K. \tag{35}$$

Multiplying by $[H]_t$, followed by transformation results in

$$(0.2\cdot[H]_t\cdot K+0.25)\cdot\frac{1}{K} \le [G]_t \le (0.8\cdot[H]_t\cdot K+4)\cdot\frac{1}{K}. \tag{36}$$

When $[H]_t \cdot K$ is set up smaller than one, the range of $[H]_t \cdot K$ is between 0 to 1. Then,

$$0.25 \le (0.2\cdot[H]_t\cdot K+0.25) \le 0.45$$

$$4 \le (0.8\cdot[H]_t\cdot K+4) \le 4.8$$

$$\therefore 0.25\cdot K_{\text{diss}} \le [G]_t \le 4.8\cdot K_{\text{diss}}. \tag{37}$$

Equation (37) clearly shows that a suitable $[G]_t$ range is connected to the magnitude of the dissociation constant K_{diss} $(= K^{-1})$. Wilcox [7] has shown also clearly the importance of the *p*-value, originally introduced by Weber [14], which is defined as

$$p = \frac{[C]}{[G]_t} \quad \text{when } [H]_t \ge [H]_t \tag{38}$$

$$p = \frac{[C]}{[H]_t} \quad \text{whn } [G]_t \ge [H]_t. \tag{39}$$

The criterion for the best condition can be written as

$$0.2 \le p \le 0.8. \tag{40}$$

Based on this criterion Equation (40), the suitable range of concentration for a titration experiment is shown as

$$\frac{1}{10}\cdot\frac{1}{K} \le [G]_t \le 10\cdot\frac{1}{K}. \tag{41}$$

This range (Equation (41)) covers the range defined by Equation (37). The concentration range of the titration experiment must be carefully chosen based on a preliminary estimation of K.

The conclusion of the concentration range of host and guest is as follows.

For a reliable experiment, the magnitude of K should be predicted, then the method decided, e.g., NMR spectroscopy or UV-visible spectroscopy or fluorometry, etc., which decides roughly the range of $[H]_t$*, and finally decide the range of* $[G]_t$ *using Figure 14 and/or Equation (36).*

Data-treatment

General view

Now how to perform the titration experiment in order to collect data for a reliable K value is known. The next step is how to treat the collected data to obtain the K value.

Some data treatment methods are generally employed. Some are approximation methods which must be employed under some premises, and some are just a regression method. Typical examples of the approximation method are Benesi and Hildebrand [15], Ketelaar [16], Nagakura and Baba [17], Scott [18], Scatchard and Hammond [19], where $[G]_t$ is used approximately instead of $[G]$.

From Equations (5) and (7) and $a = b = 1$,

$$[G]_t = [G] + K\cdot[H]\cdot[G]$$

$$\therefore [G]_t = [G](1 + K\cdot[H]). \tag{42}$$

If $K\cdot[H] \ll 1$, then it would be safely assumed that $[G]_t = [G]$. This condition is frequently encountered in weak complexation, where K is small. The condition $[G]_t \gg [H]_t$ is employed for the practical titration. Actually, an important point for this approximation is the condition $K\cdot[H] \ll 1$; nevertheless, the condition $[G]_t \gg [H]_t$ is thought to be essential. All systems cannot be investigated under this condition $[G]_t \gg [H]_t (K\cdot[H] \ll 1)$.

When the assumption $[G]_t = [G]$ cannot be applied, other approximation or regression methods have to be employed. Here the regression method is shown. Typical examples of regression methods are Rose and Drago [20], Nakano [21] and Creswell and Allred [22]. Because of the wide applicability, I decided to explain a practical guide based on the Rose–Drago method, using two examples, one for UV-visible spectroscopy and one for NMR spectroscopy.

Originally the Rose–Drago method was used for UV-visible spectroscopy for evaluating an acid-base equilibrium, the molecular addition compound of iodine. The only assumption for this original method is that there are at most two observing species which obey Beer's law in the concentration range employed. There is no other assumption. So it is widely applicable. The results are presented graphically in this method and by inspection one can quantitatively determine the precision. Firstly, an example case is described where all components are observed and overlapped, which obey Beer's law in the concentration range employed. Secondly, the way to apply this original Rose–Drago method to NMR spectroscopy, especially for the host-guest system with a fast exchange rate is described.

Rose–Drago method for UV-visible spectroscopy

Here the equilibrium of 1 : 1 host-guest complexation detected by UV-visible spectroscopy is discussed. The observed property was absorbance. The absorbance data of the titration experiment were collected. For the data-treatment of this general method, a spreadsheet program is attached as Appendix 2 [23].

$a = b = 1$ is substituted into Equation (8). Then the reciprocal is

$$\frac{1}{K} = [C] - ([H]_t + [G]_t) + \frac{[H]_t \cdot [G]_t}{[C]}. \quad (43)$$

Combining Equation (23) with Equation (43) gives

$$\frac{1}{K} = \frac{A_{\text{obs}} - \epsilon_h \cdot [H]_t - \epsilon_g \cdot [G]_t}{\epsilon_c - \epsilon_h - \epsilon_g} - ([H]_t + [G]_t) + \frac{\epsilon_c - \epsilon_h - \epsilon_g}{A_{\text{obs}} - \epsilon_h \cdot [H]_t - \epsilon_h \cdot [G]_t} \cdot [H]_t \cdot [G]_t. \quad (44)$$

This is the most complicated host-guest complexation, detecting by means of UV-visible spectroscopy because the absorption bands of all components, host, guest and complex are overlapped.

First of all, the constants ϵ_h, and ϵ_g in Equation (44) have to be obtained without a titration experiment, because they are molar absorptivities of the pure host and guest, respectively. Then it should be carried out to measure A_{obs} at different combinations of $[H]_t$ and $[G]_t$ followed by the regression of the obtained data with Equation (44). Theoretically, A_{obs} values at more than two different combinations of $[H]_t$ and $[G]_t$ give two unknowns, K and ϵ_c.

Measurement of absorbance at different combinations of $[H]_t$ and $[G]_t$ supplies the matrix $\{A_{\text{obs}n}, [H]_{tn}, [G]_{tn}\}$ consisting of 3 elements: $A_{\text{obs}n}$, observed absorbance at nth condition; $[H]_{tn}$, total concentration of host molecule at initial stage at nth condition; $[G]_{tn}$, total concentration of guest molecule at initial stage at nth condition

Combining Equation (44) and definitions (45)–(49) leads to Equation (50).

$$Y = \frac{1}{K} \quad (45)$$

$$X = \epsilon_c - \epsilon_h - \epsilon_g \quad (46)$$

$$a_n = A_{\text{obs}n} - \epsilon_h \cdot [H]_{tn} - \epsilon_g \cdot [G]_{tn} \quad (47)$$

$$b_n = [H]_{tn} + [G]_{tn} \quad (48)$$

$$c_n = \frac{[H]_{tn} \cdot [G]_{tn}}{A_{\text{obs}n} - \epsilon_h \cdot [H]_{tn} - \epsilon_g \cdot [G]_{tn}}. \quad (49)$$

Then

$$Y = \frac{a_n}{X} - b_n + c_n \cdot X. \quad (50)$$

According to Equation (50), one combination of data (e.g., $\{A_{\text{obs}1}, [H]_{t1}, [G]_{t1}\}$ and $\{A_{\text{obs}2}, [H]_{t2}, [G]_{t2}\}$) supplies a matrix of answer $\{X, Y\}$. A representative solution is as follows.

As an example, one combination of data where $n = 1$ and $n = 2$ (e.g., $\{A_{\text{obs}1}, [H]_{t1}, [G]_{t1}\}$ and $\{A_{\text{obs}2}, [H]_{t2}, [G]_{t2}\}$) is used here

$$Y = \frac{a_1}{X} - b_1 + c_1 \cdot X \quad (51)$$

$$Y = \frac{a_2}{X} - b_2 + c_2 \cdot X. \quad (52)$$

Subtracting both sides, followed by multiplying both sides by X results in

$$(c_1 - c_2) \cdot X^2 + (b_1 - b_2) \cdot X + (a_1 - a_2) = 0 \quad (53)$$

$$\therefore X = \frac{-(b_1 - b_2) \pm \sqrt{(b_1 - b_2)^2 - 4 \cdot (c_1 - c_2) \cdot (a_1 - a_2)}}{2 \cdot (c_1 - c_2)}. \quad (54)$$

Substituting Equation (51) with Equation (54) derives Y.

The obtained $\{X, Y\}$ is merely an answer which satisfies both Equations (51) and (52), but it is not the chemically correct answer. For example, chemically Y should have a positive sign. Based on such chemical limitation, correct sets of answers should be collected.

The maximum number of obtained answer pairs $\{X, Y\}$ is ${}_nC_2$ pairs for n combinations of concentration conditions. For example, 5 pairs of $\{[H]_{tn}, [G]_{tn}\}$ give 10 ($= {}_5C_2$) pairs of $\{X, Y\}$. *These $\{X, Y\}$ are obtained under the premise of 1 : 1 complexation. No approximation is introduced into this solution.* The reciprocal of the obtained Y is the binding constant K. The number of obtained K in this stage might be ${}_nC_2$.

Rose–Drago method for NMR spectroscopy

Using the equilibrium of 1 to 1 host-guest complexation as an example, the way to apply the original Rose–Drago method to NMR spectroscopy is given here. As mentioned previously, host–guest complexations are classified into two for the determination of binding constants by means of NMR spectroscopy. When the host-guest complexation equilibrium has a very slow exchange rate compared to the NMR time scale, the concentration of the complex is expressed as follows ($a = 1$ in Equation (25)).

$$[C] = \frac{n}{m + n} \cdot [H]_t. \quad (55)$$

When the host-guest complexation equilibrium has a very fast exchange rate compared to the NMR time scale, the concentration of the complex is expressed as follows ($a = 1$ in Equation (26)).

$$[C] = \frac{\delta - \delta_h}{\delta_c - \delta_h} \cdot [H]_t. \quad (56)$$

From Equations (43) and (56), the following equation is derived.

$$\frac{1}{K} = \frac{(\delta - \delta_h) \cdot [H]_t}{(\delta_c - \delta_h)} - ([H]_t + [G]_t) + \frac{(\delta_c - \delta_h)}{(\delta - \delta_h)} \cdot [G]_t. \quad (57)$$

Here we carried out the substitution using the following definitions (58)–(62).

$$Y = \frac{1}{K} \tag{58}$$

$$X = \delta_c - \delta_h \tag{59}$$

$$a_n = (\delta_n - \delta_h) \cdot [H]_{tn} \tag{60}$$

$$b_n = [H]_{tn} + [G]_{tn} \tag{61}$$

$$c_n = \frac{[G]_{tn}}{(\delta_n - \delta_h)}. \tag{62}$$

Then Equation (57) is expressed as follows.

$$Y = \frac{a_n}{X} - b_n + c_n \cdot X. \tag{63}$$

Equation (63) is the same as Equation (50). So from this stage, the same procedures for UV-visible spectroscopy can be applied for NMR spectroscopy.

Estimation of error

It is indicated in statistics that the deviation of data based on less than 30 measurements is not a normal distribution but a Student's t-distribution. So it is suitable to express the binding constant K with 95% confidence interval applied by Student's t-distribution.

Student's t-distribution includes a normal distribution. When the number of measurements is more than 30, Student's t-distribution and a normal distribution are practically the same. The actual function of Student's t-distribution is very complicated so that it is rarely used directly. A conventional way to apply Student's t-distribution is to pick up data from the critical value table of Student's t-distribution under consideration of 'degree of freedom', 'level of significance' and 'measurement data.' It is troublesome to repeat this conventional way many times. Most spreadsheet software even for personal computers has the function of Student's t-distribution. Without any tedious work, namely, picking up data from the table, a statistical treatment can be applied to experimental results based on Student's t-distribution with the aid of a computer. An application example is shown in Figure 15. When the measurement data are input into the gray parts, answers can be obtained in the cell D18 and D19 instantaneously.

When the confidence interval obtained after statistical treatment is very wide, there is high probability that a precise experiment has not been carried out. The experimental condition and also each procedure should be checked.

The data-treatment mentioned here includes no approximation, so it can be used generally. And because the required level of mathematical knowledge is not high, only a formula for polynomial of degree 2, the logical constitution can be easily understood. Moreover, the statistical treatment of the obtained data is understandable with primary statistics. These are the merits of using this method at first, in order to understand the way of determination of binding constants.

A1	B	C	D	E	F	G
2					**How to use this sheet**	
3		Data	34.72		1.Input data at D3 to D9	
4			36.84		2.input level of significance (α=5%)	
5			33.25		3.find answer in D18 and D19	
6			37.78			
7			39.16			
8			35.08			
9			34.54			
10		Average	35.91		=AVERAGE(D3:D9)	
11		s	1.93		=STDEVP(D3:D9)	
12						
13		α	0.05		0.05	
14		degree of freedom	6		=COUNTA(D3:D9)-1	
15		$t_{\alpha/2}$	2.447		=TINV(D13,D14)	
16						
17		95%	confidence interval		confidence interval	
18		K =	35.91		=AVERAGE(D3:D9)	
19		±	1.78		=D15*D11/COUNTA(D3:D9)^0.5	
20						
21						

Figure 15. Spreadsheet for statistical data-treatment based on Student's t-distribution.

When the stoichiometry of the complex is not 1 : 1 or when other premises are not satisfied, the way of data treatment should be changed or modified. A non-linear least squares data treatment is the one to be taken as one of the best approximations. Using Equations (8) and (23) for UV-visible spectroscopy or Equations (8), (25) or (26) for NMR spectroscopy, other complexations may be applied even if a and b are not one.

Non-linear least-squares method

This method is general and widely applicable but it includes an approximation. Here the equilibrium of 1 : 1 host-guest complexation detected by NMR spectroscopy is treated. The observed property is the chemical shift. The chemical shift (δ) data of the titration experiment were collected.

Equation (8), where $a = b = 1$ gives Equation (64).

$$[C] = \frac{\left([H]_t + [G]_t + \frac{1}{K}\right) \pm \sqrt{\left([H]_t + [G]_t + \frac{1}{K}\right)^2 - 4 \cdot [H]_t \cdot [G]_t}}{2}. \tag{64}$$

Equation (64) is modified with Equation (26) (rapid exchange NMR) and the following three definitions.

$$y = \delta - \delta_h \tag{65}$$

$$a = \delta_c - \delta_h \tag{66}$$

$$b = K. \tag{67}$$

Then,

$$y = a \cdot \frac{[C]}{[H]_t} = \frac{\left([H]_t + [G]_t + \frac{1}{b}\right) \pm \sqrt{\left([H]_t + [G]_t + \frac{1}{b}\right)^2 - 4 \cdot [H]_t \cdot [G]_t}}{2 \cdot [H]_t}$$

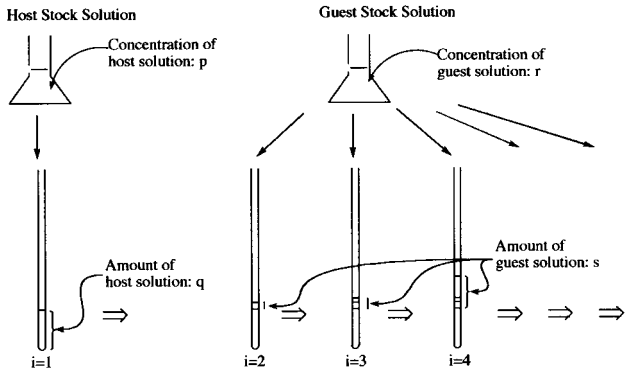


Figure 16. Illustration of typical titration experiment to show procedures and constants.

$$= \frac{a}{2}\cdot\left\{\left(\frac{[G]_t}{[H]_t}+1+\frac{1}{b\cdot[H]_t}\right)\pm\sqrt{\left(\frac{[G]_t}{[H]_t}+1+\frac{1}{b\cdot[H]_t}\right)^2-4\cdot\frac{[G]_t}{[H]_t}}\right\}. \tag{68}$$

A typical titration experiment is graphically expressed in Figure 16. The host solution in an NMR tube is titrated by the addition of guest stock solution. Equation (68) is modified with the following experimental constants and parameters according to the typical experimental method for NMR titration: p, concentration of host solution; q, amount of host solution; r, concentration of guest solution; s, amount of guest solution.

Then,

$$[H]_t = \frac{p\cdot q}{s+q} \tag{69}$$

$$[G]_t = \frac{r\cdot s}{s+q} = x \tag{70}$$

$$\therefore s = \frac{x\cdot q}{(r-x)}. \tag{71}$$

Equations (72) and (73) are derived from Equations (69)–(71).

$$[H]_t = \frac{p\cdot(r-x)}{r} \tag{72}$$

$$\frac{[G]_t}{[H]_t} = \frac{r\cdot x}{p\cdot(r-x)}. \tag{73}$$

Then basic Equation (68) is expressed with experimental constants and variables as follows.

$$y = \frac{a}{2}\cdot\left\{\left(\frac{r\cdot x}{p\cdot(r-x)}+1+\frac{r}{b\cdot p\cdot(r-x)}\right)\pm\sqrt{\left(\frac{r\cdot x}{p\cdot(r-x)}+1+\frac{r}{b\cdot p\cdot(r-x)}\right)^2-4\cdot\frac{r\cdot x}{p\cdot(r-x)}}\right\} \tag{74}$$

$$\therefore \frac{\partial y}{\partial a} = \frac{1}{2}\left\{\left(\frac{r\cdot x}{p\cdot(r-x)}+1+\frac{r}{b\cdot p\cdot(r-x)}\right)\pm\sqrt{\left(\frac{r\cdot x}{p\cdot(r-x)}+1+\frac{r}{b\cdot p\cdot(r-x)}\right)^2-4\cdot\frac{r\cdot x}{p\cdot(r-x)}}\right\} \tag{75}$$

$$\therefore \frac{\partial y}{\partial b} = \frac{a}{2}\cdot\left(-\frac{r}{b^2\cdot p\cdot(r-x)}\right)\left[1\pm\left\{\sqrt{\left(\frac{r\cdot x}{p\cdot(r-x)}+1+\frac{r}{b\cdot p\cdot(r-x)}\right)^2-4\cdot\frac{r\cdot x}{p\cdot(r-x)}}\right\}^{-0.5}\cdot\left(\frac{r\cdot x}{p\cdot(r-x)}+1+\frac{r}{b\cdot p\cdot(r-x)}\right)\right]. \tag{76}$$

The approximation procedure of this non-linear method is described below.

It is assumed a_0 and b_0 where α and β (correction of a and b) are small enough, so that y is approximately equal to Equation (80) where higher-order parts could be omitted from the Taylor series expansion (78) of y at $(a, b) = (a_0, b_0)$. Then α and β are calculated which minimize the sum of squares deviation. The following is the step-by-step procedure.

First of all, proper a_0 and b_0 are assumed.

$$\begin{cases} a = a_0 + \alpha \\ b = b_0 + \beta \end{cases} \tag{77}$$

(a, b, desired parameters; a_0, b_0, assumed parameters; α, β, correction of a, b).

Practically, the meaning of the word 'proper' here might be considered to be expressed as follows.

$$\alpha \text{ and } \beta \text{ are small enough } \left(\frac{\alpha}{a_0}\le 10^{-1}\text{and}\ \frac{\beta}{b_0}\le 10^{-1}\right).$$

Secondly, the equation of y is transferred to a linear expression by using an approximation. A Taylor series expansion of y at $(a, b) = (a_0, b_0)$ is the following Equation (78).

$$y = y_0 + \left(\frac{\partial y}{\partial a}\right)_0\cdot\alpha + \left(\frac{\partial y}{\partial b}\right)_0\cdot\beta + \left(\frac{\partial^2 y}{\partial a^2}\right)_0\cdot\alpha^2 + \left(\frac{\partial^2 y}{\partial b^2}\right)_0\cdot\beta^2 + \cdots. \tag{78}$$

The values, y_0, $(\partial y/\partial a)_0$, and $(\partial y/\partial b)_0$ are obtained based on the above-mentioned Equations (74)–(76) where $a = a_0$, $b = b_0$. Approximately the higher order parts can be replaced with zero, because α and β are small enough by the assumption.

$$\left(\frac{\partial^2 y}{\partial a^2}\right)_0\cdot\alpha^2 + \left(\frac{\partial^2 y}{\partial b^2}\right)_0\cdot\beta^2 + \cdots \approx 0. \tag{79}$$

Then the following Equation (80) after approximation is derived from Equations (78) and (79).

$$y \approx y_0 + \left(\frac{\partial y}{\partial a}\right)_0\cdot\alpha + \left(\frac{\partial y}{\partial b}\right)_0\cdot\beta. \tag{80}$$

The next step is to minimize the sum of squares deviations for titration data, by determining proper α and β. Using Equation (80), deviation d_i is defined as follows for each titration (I = 1, 2, 3, ...), where i is the running index.

$$d_I = y_I - y_{0i} - \left(\frac{\partial y}{\partial a}\right)_{0i} \cdot \alpha - \left(\frac{\partial y}{\partial b}\right)_{0i} \cdot \beta. \qquad (81)$$

The value y_i is obtained from Equation (65) for each titration (i = 1, 2, 3, ...). The values, y_{0i}, $(\partial y/\partial a)_{0i}$, and $(\partial y/\partial b)_{0i}$ are obtained based on the above-mentioned Equations (74)–(76), where $a = a_0$, $b = b_0$, and constants (p, q, r, s) for each titration (i = 1, 2, 3, ...)

In order to obtain the value α and β where the sum of the squares deviation ($\sum d_i^2$) is minimized, the following equations are the necessary conditions.

$$\frac{\partial}{\partial \alpha} \sum d_i^2 = 0 \qquad (82)$$

$$\frac{\partial}{\partial \beta} \sum d_i^2 = 0. \qquad (83)$$

The d_i is the function of y_i, y_0, $(\partial y/\partial a)_0$, $(\partial y/\partial b)_0$, α and β. The values y_i, y_0, $(\partial y/\partial a)_0$, and $(\partial y/\partial b)_0$ are obtained from Equations (65), (74)–(76) so that Equations (82) and (83) are expressed as functions of two parameters (α, β). The two equations with two parameters (α, β) are easily solved and give only one pair of answers (α, β). When the obtained values of α, β are small enough ($\alpha/a_0 \leq 10^{-3}$ and $\beta/b_0 \leq 10^{-3}$), the assumed a_0 and b_0 are considered to have been proper. Consequently, $a = a_0 + \alpha$ and $b = b_0 + \beta$ are thought to be what was desired to be determined. When α and β are not small enough, the assumption of proper a_0 and b_0 must be repeated until small enough α and β are obtained.

The practically important point here is how to assume proper a_0 and b_0. Theoretically, there is no general rule on how to assume proper a_0 and b_0. A generally recommended way is as follows. First of all, plausible a_0 and b_0 may be used, based on the information of similar experimental results; then the sum of obtained α and a_0 may be used as a_0, sum of β and b_0 as b_0 for the second trial. This trial is repeated until the small enough α and β are obtained. One other way is shown in Appendix 3 [23], which requires only few repetitions. Another easy way is to use the software function, e.g., SOLVER [24], to minimize $\sum d_i^2$ by changing a_0 and b_0. In any event, the finally obtained α and β give δ_c and K using Equations (66) and (67).

The above-mentioned non-linear least squares method for the case with two parameters (α, β) is the basic one and easily extended to the cases with more parameters. Considering the possibility of obtaining the reliable δ_h of this NMR titration experiment, data-treatment should be carried out with three parameters for better regression. The programs of spreadsheet software for this three-parameters-method are developed and shown in Appendix 4 [25].

Summary and concluding remarks

Finally, this article is concluded by showing our research result as an impressive example, obtained by determining the binding constant. The binding constants of chiral crown ethers **5**, **6** and **7**, with both enantiomers of 2-amino-1-propanol were determined at various temperatures. The obtained results are summarized in Figure 17, where $\Delta\Delta G = -RT \ln(K_S/K_R)$. A normal temperature dependence is exemplified in the case of **5**, where raising the temperature results in decreasing enantioselectivity. On the other hand, in the cases of **6** and **7**, (R)-selectivity ($\Delta\Delta G > 0$) at low temperature (<280 K) decreases with rising temperature until it reaches 280 K. It is non-enantioselective at 280 K, which is called the isoenantioselective temperature. Continuous temperature elevation results in an inversion of enantioselectivity and in an increasing (S)-selectivity ($\Delta\Delta G < 0$). This is the first observation of the inversion of enantioselectivity by temperature control in the complexation of a chiral crown ether with a chiral amine [26]. The determination of a binding constant at various temperatures would be a useful way to reveal the effective criteria for molecular design under consideration of an entropy effect together with an enthalpy effect.

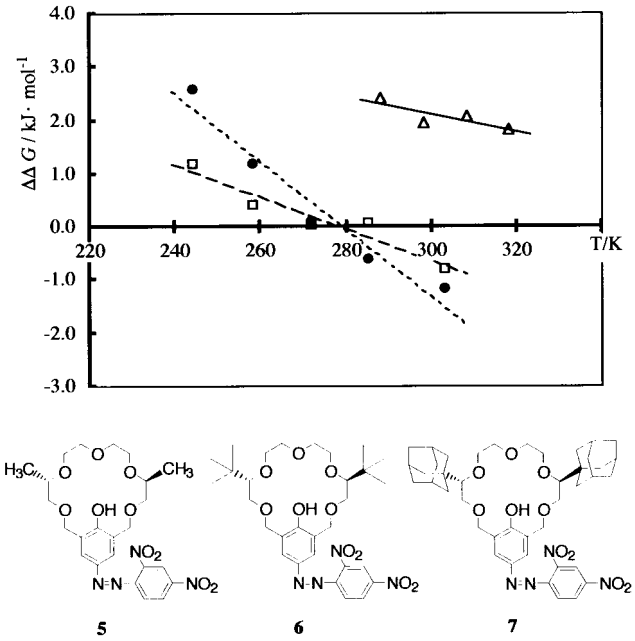


Figure 17. Temperature dependence of $\Delta\Delta G(= \Delta G_S - \Delta G_S)$ for the complexation of crown ethers **5**-**7** (for (S, S)-**5**, △; (S, S)-**6**, □; (S, S)-**7**, ●) with 2-amino-1-propanol in chloroform.

For an understanding of the basic theoretical principle, a practical measurement and also a practical data-treatment of an experiment to determine a binding constant, this article is described here in detail using a basic level of mathematics, statistics, and programs of spreadsheet software. It is believed the programs attached as appendices would function with commonly available spreadsheet software on personal computers and provide another way to understand the contents described in this article. Moreover, the appendixes are useful for actual experiment. It is hoped the style of this article is one of the better ways at this time to provide to chemists, information on how to determine binding constants.

Acknowledegments

The author acknowledges Prof. K. Naemura and Prof. Y. Tobe for their encouragement and helpful discussions. The authors' research in this field has been supported partially by SUNBOR foundation from Suntory Institute for Bioorganic Research and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

Appendix

Appendix-1 Spreadsheet for Continuous Variation Method by UV-visible Spectroscopy

0. Input data into gray cells and follow the instructions below

1. Preparation of host stock solution and measure absorbance

Host	WCR1
Weight(mg)	7.85 mg
Molecular Weight	710.74
Volume(ml)	25.0 ml

Host Stock Soln. 4.42E-04 mol/l → λobsv 576 nm; Absorbance at 576nm is 0.051

2. Preparation of guest stock solution

Guest	2APO
Weight(mg)	6.88 mg
Purity(%)	100%
Molecular Weight	61.08
Volume(ml)	10.0 ml

picked up 0.98 ml → diluted to 25.0 ml → Absorbance at 576nm is 0.029

Guest Stock Soln. 4.42E-04 mol/l

3. Input the amount of host and guest stock solution mixed in UV cell and observed absorbance *Aobs*

					ε h		ε g	Ka	ε c		average
	Added amount of stock soln.				115.0		65.678	266	38000		4.42E-04
Run	Host(ml)	Guest(ml)	Aobs	[H]	Ah parts	[G]	Ag parts	forecasted[C]	forecasted Ac	forecasted Aobs	[H]+[G]
1	3.5	0.5	0.2500	3.87E-04	0.0445	5.52E-05	3.63E-03	5.68E-06	0.2157	0.2657	4.42E-04
2	3.2	0.8	0.3568	3.53E-04	0.0406	8.83E-05	5.80E-03	8.30E-06	0.3155	0.3619	4.42E-04
3	3.0	1.0	0.3944	3.31E-04	0.0381	1.10E-04	7.25E-03	9.73E-06	0.3697	0.4151	4.42E-04
4	2.5	1.5	0.5028	2.76E-04	0.0318	1.66E-04	1.09E-02	1.22E-05	0.4621	0.5048	4.42E-04
5	2.0	2.0	0.5267	2.21E-04	0.0254	2.21E-04	1.45E-02	1.30E-05	0.4929	0.5328	4.42E-04
6	1.5	2.5	0.5027	1.66E-04	0.0191	2.76E-04	1.81E-02	1.22E-05	0.4621	0.4993	4.42E-04
7	1.0	3.0	0.4136	1.10E-04	0.0127	3.31E-04	2.18E-02	9.73E-06	0.3697	0.4042	4.42E-04
8	0.8	3.2	0.3723	8.84E-05	0.0102	3.53E-04	2.32E-02	8.30E-06	0.3155	0.3488	4.42E-04
9	0.5	3.5	0.2400	5.52E-05	0.0064	3.86E-04	2.54E-02	5.68E-06	0.2157	0.2474	4.42E-04

4. For the calcd. line in Figure, do Tool-Solver: minimize E34 by changing I19&J19

Graph Data

Run	[H]t/([H]t+[G]t)	forecasted Ac	Aobs-Ah-Ag	Σdi^2
	1.00	0.0000	0.0000	1.39E-03
1	0.88	0.2157	0.2019	1.89E-04
2	0.80	0.3155	0.3104	2.63E-05
3	0.75	0.3697	0.3490	4.27E-04
4	0.63	0.4621	0.4602	3.87E-06
5	0.50	0.4929	0.4868	3.78E-05
6	0.38	0.4621	0.4655	1.15E-05
7	0.25	0.3697	0.3791	8.91E-05
8	0.20	0.3155	0.3389	5.50E-04
9	0.13	0.2157	0.2083	5.46E-05
	0.00	0.0000	0.0000	

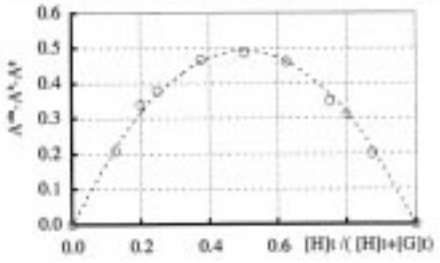


Figure Modified Job's Plot for Complexation of Host and Guest by UV-visible spectroscopy
○ : observed, ------- : calculated

Appendix-2 Spreadsheet for Rose-Drago Method

by UV-visible Spectroscopy

Instruction

1. Input data into gray cells at 2!
2. Remove improper data at 8!
3. Check reliability in the graph at 9!
4. That's all!

1. Basic Equations

$$1/K=(An\text{-}Ahn\text{-}Agn)(\varepsilon_c-\varepsilon_h-\varepsilon_g)-([H]tn+[G]tn)$$
$$+[H]tn[G]tn(\varepsilon_c-\varepsilon_h-\varepsilon_g)/(An\text{-}Ahn\text{-}Agn)$$

$an = An\text{-}Ahn\text{-}Agn$

$bn = -([H]tn+[G]tn)$

$cn = [H]tn[G]tn/(An\text{-}Ahn\text{-}Agn)$

$An\text{-}\varepsilon_a = [H]tn\text{-}\varepsilon_g - [G]tn$

$Y = 1/K$

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

$\therefore Y = an/X + bn + cn*X$

2. Data of Titration Experiment

Host Abb.	SR(24D)		[H]tn	[G]tn	An	Ahn	Agn	An-Ahn-Agn
Guest Abb.	IAPO	n=1	1.88E-05	2.15E-02	0.6477	1.88E-05	2.15E-02	0.626
Temp/℃	30	n=2	1.88E-05	1.62E-02	0.6026	1.88E-05	1.62E-02	0.586
lobs/nm	580	n=3	1.88E-05	1.08E-02	0.5376	1.88E-05	1.08E-02	0.527
ε_h	1	n=4	1.88E-05	7.54E-03	0.4700	1.88E-05	7.54E-03	0.462
ε_g	1	n=5	1.88E-05	4.31E-03	0.3554	1.88E-05	4.31E-03	0.351

3. Calculation of *an*, *bn*, and *cn* .

4. Calod. X1, X2

5. Ordering of X1, X2(X1>X2)

n	an	bn	cn	combination of n		X1	X2	X1-X2	X1	X2
n=1	0.6261	-2.16E-02	6.48E-07	1	2	7.37E+00	4.17E+04	-4.17E+04	4.17E+04	7.37E+00
n=2	0.5864	-1.62E-02	5.19E-07	2	3	1.11E+01	4.02E+04	-4.02E+04	4.02E+04	1.11E+01
n=3	0.5268	-1.08E-02	3.85E-07	3	4	1.99E+01	4.14E+04	-4.14E+04	4.14E+04	1.99E+01
n=4	0.4624	-7.56E-03	3.07E-07	4	5	3.45E+01	4.25E+04	-4.25E+04	4.25E+04	3.45E+01
n=5	0.3511	-4.33E-03	2.31E-07	5	1	1.60E+01	4.13E+04	-4.13E+04	4.13E+04	1.60E+01
n=1	0.6261	-2.16E-02	6.48E-07	1	3	9.22E+00	4.10E+04	-4.10E+04	4.10E+04	9.22E+00
n=2	0.5864	-1.62E-02	5.19E-07	2	4	1.44E+01	4.07E+04	-4.07E+04	4.07E+04	1.44E+01
n=3	0.5268	-1.08E-02	3.85E-07	3	5	2.72E+01	4.20E+04	-4.19E+04	4.20E+04	2.72E+01
n=4	0.4624	-7.56E-03	3.07E-07	4	1	1.17E+01	4.11E+04	-4.11E+04	4.11E+04	1.17E+01
n=5	0.3511	-4.33E-03	2.31E-07	5	2	1.99E+01	4.12E+04	-4.11E+04	4.12E+04	1.99E+01
									4.13E+04	1.71E+01

6. Data for Graph

Y=1/K	X=εc-εh-εg									
Y=an/X+bn+cnX	50	1.2E+02	2.9E+02	7.1E+02	1.7E+03	4.2E+03	1.0E+04	2.5E+04	4.2E+04	6.0E+04
n=1	-9.0E-03	-1.6E-02	-1.9E-02	-2.0E-02	-2.0E-02	-1.9E-02	-1.5E-02	-5.5E-03	5.9E-03	1.7E-02
n=2	-4.4E-03	-1.1E-02	-1.4E-02	-1.5E-02	-1.5E-02	-1.4E-02	-1.1E-02	-3.3E-03	5.8E-03	1.5E-02
n=3	-2.4E-04	-6.4E-03	-8.9E-03	-9.8E-03	-9.8E-03	-9.0E-03	-6.8E-03	-1.2E-03	5.5E-03	1.2E-02
n=4	1.7E-03	-3.7E-03	-5.9E-03	-6.7E-03	-6.8E-03	-6.2E-03	-4.4E-03	5.2E-05	5.5E-03	1.1E-02
n=5	2.7E-03	-1.4E-03	-3.1E-03	-3.7E-03	-3.7E-03	-3.3E-03	-1.9E-03	1.4E-03	5.5E-03	9.5E-03

7. Y1, Y2 and *K1*, *K2* from X1, X2

Y1	Y2	K 1=1/Y1	K 2=1/Y2
5.49E-03	6.34E-02	1.82E+02	1.58E+01
4.72E-03	3.68E-02	2.12E+02	2.72E+01
5.17E-03	1.57E-02	1.93E+02	6.39E+01
5.51E-03	5.86E-03	1.82E+02	1.71E+02
5.23E-03	1.77E-02	1.91E+02	5.66E+01
5.00E-03	4.63E-02	2.00E+02	2.16E+01
4.94E-03	2.46E-02	2.02E+02	4.07E+01
5.38E-03	8.58E-03	1.86E+02	1.17E+02
5.06E-03	3.20E-02	1.98E+02	3.13E+01
5.19E-03	1.33E-02	1.93E+02	7.49E+01
		1.94E+02	6.19E+01

8. Statistical treatment of X1(εc-εh-εg) and *K*

combination of n		X1 Data	Check Data Here	K1=1/Y1 Data	Check Data Here
1	2	4.17E+04	4.17E+04	1.82E+02	1.82E+02
2	3	4.02E+04	4.02E+04	2.12E+02	2.12E+02
3	4	4.14E+04	4.14E+04	1.93E+02	1.93E+02
4	5	4.25E+04	4.25E+04	1.82E+02	1.82E+02
5	1	4.13E+04	4.13E+04	1.91E+02	1.91E+02
1	3	4.10E+04	4.10E+04	2.00E+02	2.00E+02
2	4	4.07E+04	4.07E+04	2.02E+02	2.02E+02
3	5	4.20E+04	4.20E+04	1.86E+02	1.86E+02
4	1	4.11E+04	4.11E+04	1.98E+02	1.98E+02
5	2	4.12E+04	4.12E+04	1.93E+02	1.93E+02
	Average	41312	41312	194	194
	standard deviation	619	619	9	9
evel of significance α		0.05	0.05	0.05	0.05
	degree of freedom	9	9	9	9
	$t_{\alpha/2}$	2.262	2.262	2.262	2.262
confidence interval	95%			95%	95%
	x	41312	41312	194	K=193.9
	±	443	443	6	±6.4
	Upper limit	41755	41755	200	200
	Lower limit	40870	40870	187	187

ε_c= 41314 ±443

9. Graphical Expression

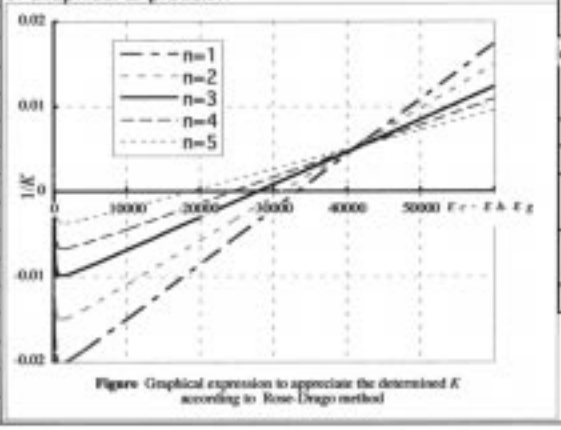
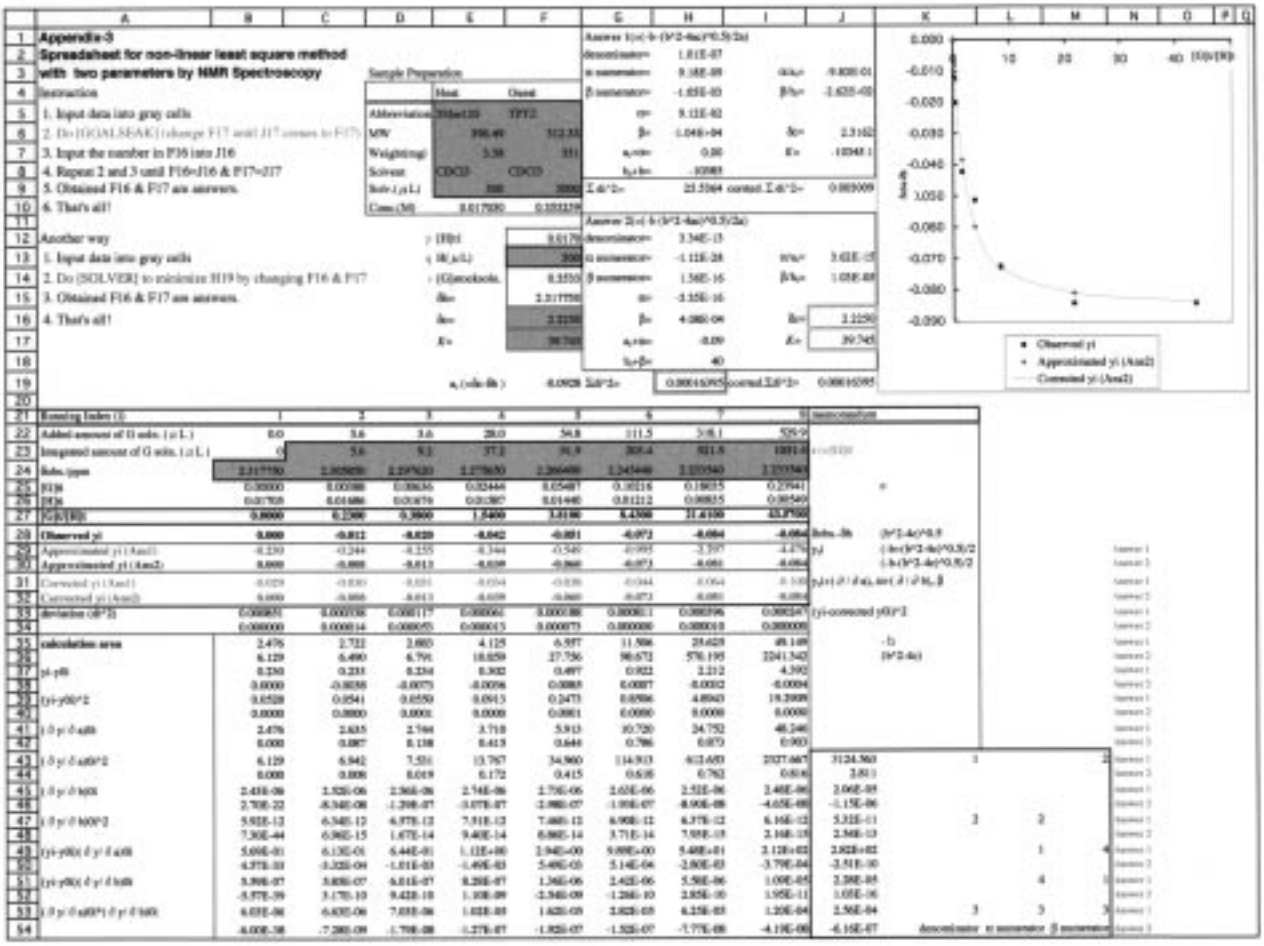
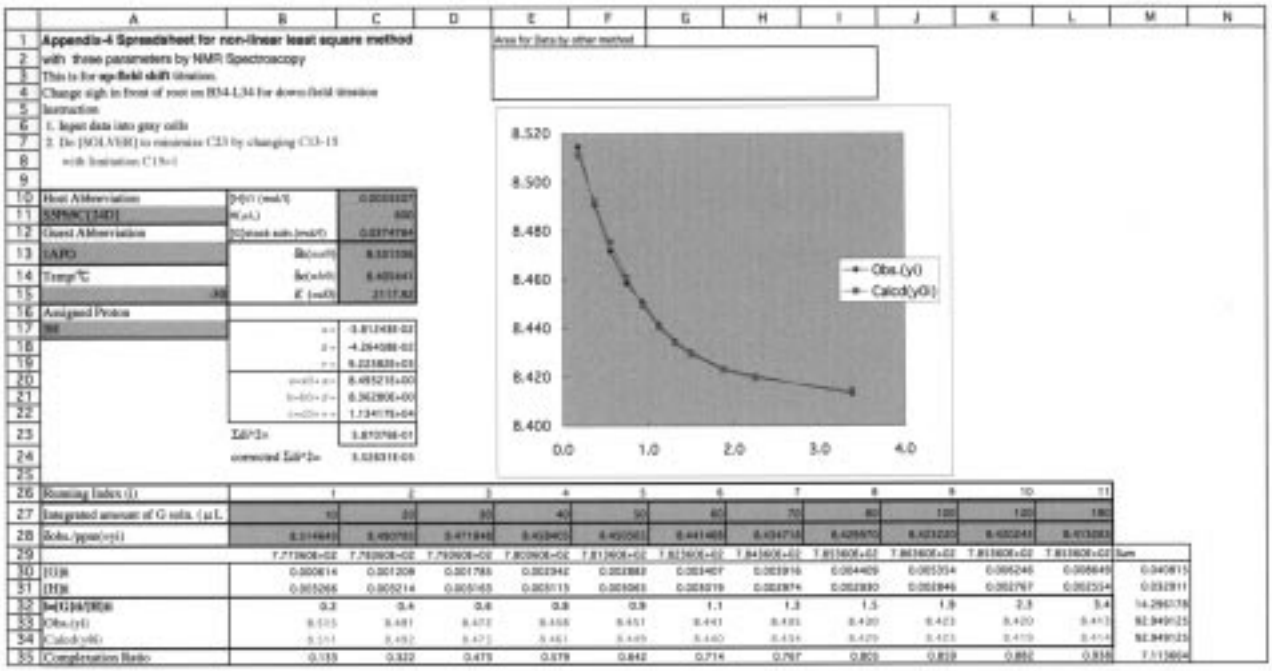


Figure Graphical expression to appreciate the determined *K* according to Rose-Drago method

Figure



Figure



Figure

References

1. For a chirality indicator, see: G. Hollmann and F. Vögtle: *Chem. Ber.* **117**, 1355 (1984); T. Kaneda, K. Hirose, and S. Misumi: *J. Am. Chem. Soc.* **111**, 742 (1989); S. Misumi: *Kagaku*, No.5, opening color pages (1987); Y. Kubo, S. Maeda, S. Tokita, and M. Kubo: *Nature* **382**, 522 (1996); K. Naemura, K. Ogasahara, K. Hirose, and Y. Tobe: *Tetrahedron: Asymmetry* **8**, 19 (1997); K. Ogasahara, K. Hirose, Y. Tobe, and K. Naemura: *J. Chem. Soc., Perkin Trans. 1* 3227 (1997).
2. For a FAB Mass EL method, see: M. Sawada, Y. Okumura, M. Shizuma, Y. Takai, Y. Hidaka, H. Yamada, T. Tanaka, T. Kaneda, K. Hirose, S. Misumi, and S. Takahashi: *J. Am. Chem. Soc.* **115**, 7381 (1993); M. Sawada, Y. Takai, H. Yamada, T. Kaneda, K. Kamada, T. Mizooku, K. Hirose, Y. Tobe, and K. Naemura: *J. Chem. Soc., Chem. Commun*. 2497 (1994); M. Sawada, Y. Takai, H. Yamada, S. Hirayama, T. Kaneda, T. Tanaka, K. Kamada, T. Mizooku, S. Takeuchi, K. Ueno, K. Hirose, Y. Tobe, and K. Naemura: *J. Am. Chem. Soc.* **117**, 7726 (1995); M. Sawada, Y. Takai, T. Kaneda, R. Arakawa, M. Okamoto, H. Doe, T. Matsuo, K. Naemura, K. Hirose, and Y. Tobe: *J. Chem. Soc., Chem. Commun*. 1735 (1996); M. Sawada, Y. Takai, H. Yamada, J. Nishida, T. Kaneda, R. Arakawa, M. Okamoto, K. Hirose, T. Tanaka, and K. Naemura: *J. Chem. Soc., Perkin Trans. 2* 701 (1998).
3. X. X. Zhang, J. S. Bradshaw, and R. M. Izatt: *Chem. Rev.* **97**, 3313 (1997).
4. R. M. Izatt, K. Pawlak, and J. S. Bradshaw: *Chem. Rev.* **91**, 1721 (1991).
5. R. M. Izatt, J. S. Bradshaw, K. Pawlak, R. L. Bruening, and B. J. Tarbet: *Chem. Rev.* **92**, 1261 (1992).
6. K. A. Connors: *Binding Constants*, John-Wiley & Sons, New York (1987).
7. C. S. Wilcox: 'Design, synthesis, and evaluation of an efficacious functional group dyad. Methods and limitations in the use of NMR for measuring host-guest interactions', in H.-J. Schneider and H. Dürr (eds.), *Frontiers in Supramolecular Organic Chemistry and Photochemistry*, VCH Verlag, Weinheim (1991), pp. 123.
8. H. Tsukube *et al*.: 'Determination of stability constants', in J. E. D. Davies and J. A. Ripmeester (eds.), *Comprehensive Supramolecular Chemistry*, Vol. 8, Elsevier Science, (1996), pp 425–482.
9. Author developed the programs for determination of binding constants of 1 : 1 host-guest complexes using the spreadsheet software on a personal computer (Microsoft Excel version 5.0). All programs attached as appendixes are developing and the latest ones will be published elsewhere.
10. Y. Shibata, B. Inoue, and Y. Nakatuka, *Nihon Kagakukaishi* **42**, 983 (1921); R. Tsuchida: *Bull. Chem. Soc. Japan* **10**, 27 (1935); P. Job: *Compt. Rend.* **180**, 928 (1925): *Ann. Chim. Phys*. **9**, 113 (1928).
11. A. E. Harvey and D. L. Manning: *J. Am. Chem. Soc.* **72**, 4488 (1950): H. E. Bent and C. L. French: *J. Am. Chem. Soc.* **63**, 568 (1941).
12. J. H. Yoe and A. L. Jones: *Ind. Eng. Chem. Anal. Ed.* **16**, 111 (1944).
13. K. Hirose: unpublished software. Simple instruction of this software is shown on the sheet.
14. G. Weber: in B. Pullman and M. Weissbluth (eds.), *Molecular Biophysics*, Academic Press, New York (1965), pp. 369–397.
15. H. A. Benesi and J. H. Hildebrand: *J. Am. Chem. Soc.* **71**, 2703 (1949).
16. J. A. A. A. Ketelaar, C. van de Stolpe, A. Goudsmit, and W. Dzcubas: *Rec. Trav. Chim* **71**, 1104 (1952).
17. S. Nagakura: *J. Am. Chem. Soc.* **76**, 3070 (1954); *J. Am. Chem. Soc.* **80**, 520 (1958); H. Baba: *Bull. Chem. Soc. Japan* **31**, 169 (1958).
18. R. L. Scott: *Rec. Trav. Chim. Pays-Bas* **75**, 787 (1956).
19. P. R. Hammond: *J. Chem. Soc.* **1964**, 479.
20. N. J. Rose and R. S. Drago: *J. Am. Chem. Soc.* **81**, 6138 (1959): S. Nagakura: *J. Am. Chem. Soc.* **80**, 520 (1958).
21. M. Nakano, N. I. Nakano, and T. Higuchi: *J. Phys. Chem.* **71**, 3954 (1967).
22. C. J. Creswell and A. L. Allred: *J. Phys. Chem.* **66**, 1469 (1962): H. Stamm, W. Lamberty, and J. Stafe: *Tetrahedron* **32**, 2045 (1976).
23. K. Hirose: unpublished software. Simple instruction of this software is shown on the spreadsheet.
24. Add-in function of spreadsheet software on a personal computer (Microsoft Excel ver. 5.0).
25. K. Hirose, Y. Nishikawa, and K. Matsunaga: unpublished software. Simple instruction of this software is shown on the spreadsheet.
26. For the temperature-dependent inversion of enantioselectivity, see: K. Hirose, J. Fuji, K. Kamada, Y. Tobe, and K. Naemura: *J. Chem. Soc., Perkin Trans. 2* 1649 (1997); Y. Inoue, T. Yokoyama, N. Yamasaki, and A. Tai: *Nature* (London) **341**, 225 (1989); K. Watabe, R. Charles, and E. Gil-Av: *Angew. Chem. Int. Ed. Engl.* **28**, 192 (1989); V. Schurig, J. Ossig, and R. Link: *Angew. Chem. Int. Ed. Engl.* **28**, 194 (1989); V. T. Pham, R. S. Phillips, and L. G. Ljungdahl: *J. Am. Chem. Soc.* **111**, 1935 (1989). For the temperature-dependent enantioselectivity of crown ether with amine, see: K. Naemura; J. Fuji; K. Ogasahara; K. Hirose, and Y. Tobe: *Chem. Commun*. 2749 (1996); K. Naemura, T. Wakebe, K. Hirose, and Y. Tobe: *Tetrahedron: Asymmetry* **8**, 2585 (1997); K. Naemura, K. Matsunaga, J. Fuji, K. Ogasahara, Y. Nishikawa, K. Hirose, and Y. Tobe, *Anal. Science* **14**, 175 (1998); K. Naemura, K. Nishioka, K. Ogasahara, Y. Nishikawa, K. Hirose, and Y. Tobe: *Tetrahedron: Asymmetry*, **9**, 563 (1998).