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Improved Accuracy and Efficiency in the Determination of Association Constants with the Spectrophotometric Method

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Abstract

A new method is proposed for measuring association constants and has been used to study the complexation of β-cyclodextrin with nine substituted benzenes. Computer simulation was conducted to compare the performance of the new method with that of the Benesi–Hildebrand method. It showed that the accuracy and success rate of the new method were both higher than those of the BH method. Hence, the new method is a recommended approach for a convenient and reliable measurement of association constants.

Introduction

The association of a host molecule such as cyclodextrin (CD) [1] with a guest substrate is key to supramolecular chemistry. The association constants can be measured with various spectroscopic methods [2–7], in which the Benesi–Hildebrand (BH) method [8–11] is usually used to treat the data.

However, the BH method was often found inaccurate or even unreliable in estimating the association constants [11–16], though whether the inaccuracy results from the intercept [17] or slope [18, 19] of the regression remains unclear. Some authors regarded the inaccuracy as the result of the difficulty in measuring the association constant and molar absorption constant independently [20, 21]. Less accurate estimation of the absorption constant would cause less accurate estimation of the association constant [17]. Others were critical that the BH method placed more emphasis on lower concentration values than on higher ones [12], thus the slope of the regression was too sensitive to the former ones [11]. A small error in the concentration of the host or guest was found to bring about a large error in the association constant [22].

Modifications were proposed for the BH method. Sometimes, the association constant could be obtained independently of the molar absorption [23]. A graphical procedure [18, 19, 24, 25], which used several values of the molar absorption constants for a given set of experimental data and thus generated an averaged association constant, was recommended. A cubic equation was also formulated for the association constant, which could be solved by a root determination for real polynomials [26]. Recently, some nonlinear regression methods were developed which used the association constant determined from the BH method as the seed value for the fits [11, 27–29]. However, it was suspected that the use of nonlinear least-square regressions usually did not alter the original value of the association constant from the BH method by more than $\pm 5 \sim 15\%$ [12].

Nevertheless, a clear drawback of the BH method is its requirement of very unequal host and guest concentrations [13, 30, 31]. Although this requirement allows for mathematical simplicity, it can often make the monitored signal too weak to be observable [32]. Thus the spectroscopic parameters and association constants obtained *via* the BH method are often found difficult to reproduce [30–33]. Sometimes, the association constants could not even be estimated due to the small and inconsistent changes in the absorption spectra [34]. Therefore, a more reliable and convenient method is still required for measuring the association constants [35].

Herein, a new method is proposed for determining the association constant, especially that in CD inclusion complexation. Computer simulation was performed to compare the performance of the BH method with that of the new method in detail.

Theory

Benesi–Hildebrand method

Generally, CD forms 1:1 complexes with the substrate [1]. The association constant is

$$K = \frac{[S \cdot CD]}{[CD][S]} = \frac{X_i}{(C_{CD} - C_S X_i)(1 - X_i)} \qquad (1)$$

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in which $[S \cdot CD]$, $[CD]$ and $[S]$ represent the equilibrium concentrations of the complexed substrate, free CD and free substrate, respectively. C_{CD} and C_S stand for the analytical concentrations of the CD and substrate, and $X_i = [S \cdot CD]/C_S$.

Assume that the molar absorption constants of the free and bound substrate at a certain wavelength are ϵ_0 and ϵ_∞, respectively. (Herein, the absorption of CD is presumably zero [2, 5].) Thus, when $C_{CD} = 0$, the absorption of the solution is $A_0 = \epsilon_0 l C_S$, in which l is the thickness of the sample. When the analytical concentration of CD is C^i_{CD}, the apparent absorption of the solution is $A_i = \epsilon_0 l C_S(1 - X_i) + \epsilon_\infty l C_S X_i$. Suppose that C_S is constant, then $A_i = A_0 + \Delta A X_i$, in which $\Delta A = (\epsilon_\infty - \epsilon_0) l C_S$. If $C^i_{CD} \gg C_S$, the following equation can be obtained,

$$\frac{1}{K} = \frac{(C^i_{CD} - C_S X_i)(1 - X_i)}{X_i} = C^i_{CD}\frac{(1 - X_i)}{X_i}$$

$$= C^i_{CD}\left(\frac{\Delta A}{\Delta A_i} - 1\right), \quad (2)$$

where $\Delta A_i = A_i - A_0 = \Delta A X_i$. Rearranging Equation (2) gives

$$\frac{1}{\Delta A_i} = \frac{1}{\Delta A} + \frac{1}{\Delta A K}\frac{1}{C^i_{CD}}. \quad (3)$$

Hence, plotting $1/\Delta A_i$ *vs.* $1/C^i_{CD}$ gives a slope of $1/(\Delta A \cdot K)$ and an intercept of $1/\Delta A$. The ratio of the intercept to the slope can be taken as an estimation of the association constant K.

The new method

An alternative way to estimate the association constant is based on the following equation

$$\frac{C^1_{CD}}{X_1} - C^1_{CD} + C_S X_1 = \frac{1}{K} = \frac{C^2_{CD}}{X_2} - C^2_{CD} + C_S X_2. \quad (4)$$

Since $X_i = \Delta A_i/\Delta A$, solving Equation (4) yields,

$$\Delta A = \frac{(C^1_{CD} - C^2_{CD})\Delta A_1 \Delta A_2 - \sqrt{(C^1_{CD} - C^2_{CD})^2 \Delta A_1^2 \Delta A_2^2 - 4(C^1_{CD}\Delta A_2 - C^2_{CD}\Delta A_1) C_S (\Delta A_1 - \Delta A_2)\Delta A_1 \Delta A_2}}{2(C^1_{CD}\Delta A_2 - C^2_{CD}\Delta A_1)}. \quad (5)$$

Herein, the other root of the equation is rejected since it will make X_1 and X_2 not fall into the range of (0, 1) [36]. Substituting Equation (5) in Equation (2) gives

$$K = \frac{X_1}{(C^1_{CD} - C_S X_1)(1 - X_1)} = \frac{\Delta A \Delta A_1}{(C^1_{CD}\Delta A - C_S \Delta A_1)(\Delta A - \Delta A_1)}. \quad (6)$$

Thus, if A_0, A_1, A_2, C_S, C^1_{CD} and C^2_{CD} are known, the association constant can be easily generated. It is noteworthy that here the requirement $C^i_{CD} \gg C_S$ is not needed. In addition, desirable CD concentrations can be chosen according to experience or computer simulation as shown later.

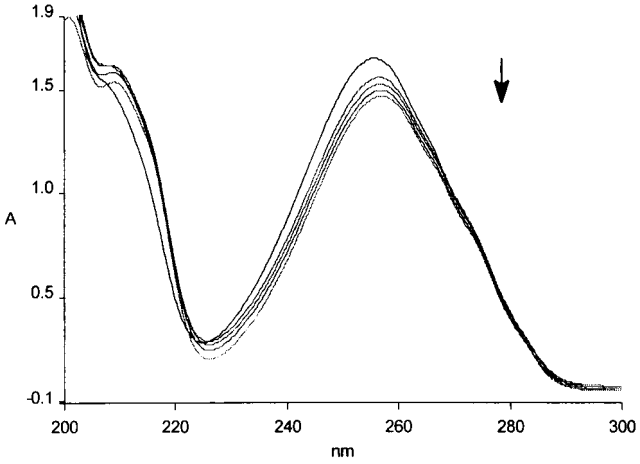

Figure 1. The effect of adding β-CD on the UV-visible absorption of methyl *p*-hydroxybenzoate.

Experimental

Methods

The absorption spectra were measured with a Perkin Elmer Spectrometry Lambda Bio20 spectrophotometer. β-CD and the substituted benzenes were of the best available grade. Doubly distilled water was used through out the experiment.

The complexation of β-CD with methyl *p*-hydroxybenzoate (MHB) was studied in detail. In the BH method, the change in absorption was measured as a function of β-CD concentration (see Figure 1). The concentration of MBH in a phosphate buffer (pH = 7) was held constant at 1.06×10^{-4} M for all the solutions. The concentration of β-CD was chosen as 0.00, 2.38, 3.56, 4.75, 5.94, 7.13, 8.32 and 9.50 mM, respectively. Three replicate solutions were prepared for every concentration of β-CD. After ultrasonification for 10min, the solutions were allowed to stand for several hours before measurement at room temperature. The absorption values of the three solutions with the same concentrations of β-CD were measured independently and then averaged to yield the absorption value at the given host concentration.

In the new method, the concentration of MHB in a phosphate buffer (pH = 7) was also held constant at 1.05×10^{-4} M for all the solutions. The concentration of β-CD was chosen as 1.19 and 9.51 mM. Four replicate solutions were prepared for every concentration of β-CD. Their absorption values were measured independently and then averaged to yield the absorption value at the given host concentration. The standard deviation of the absorption values of the four replicate solutions was also calculated. It was converted to the relative standard deviation (*rsd*) when divided by the average absorption value at the same host concentration.

Table 1. The association constants of β-CD complexation with eight substituted benzenes measured by the new method

Guest compound	Wavelength (nm)	ϵ_0/1000 (m^2/mol)	ϵ_∞/1000 (m^2/mol)	C_S (10^{-4}M)	C^1_{CD} (10^{-3}M)	C^2_{CD} (10^{-3}M)	K (M^{-1})	K (literature)[a] (M^{-1})
Benzoic acid	230	1.15	1.05	1.75	1.19	9.52	1153	370, 338, 590, 632, 794, 357, 1828, 546, 1380, 126, 1230[b]
4-Nitro benzoic acid	264	1.13	0.98	1.04	1.19	8.30	296	220
Benzaldehyde	250	1.34	1.25	0.99	2.37	8.30	148	1790, 1640, 150[b]
4-Nitro benzaldehyde	268	1.33	1.14	1.12	3.57	9.52	104	–
Phenol	211	0.48	0.43	1.52	3.54	9.41	72	94, 102, 18.9, 2500, 129, 40, 95[b]
4-Nitro phenol	318	0.96	0.90	1.28	1.19	9.52	288	314, 260, 230, 407, 1000, 201, 301, 130, 1150
Aniline	230	0.78	0.74	2.2	2.37	8.30	76	125, 56, 50, 86[b]
4-Nitro aniline	381	1.30	1.39	0.811	2.36	9.41	307	260, 322, 300, 2045

[a] The associations constants are taken from Ref. 1.
[b] The association constants are taken from Ref. 39.

In addition to MHB, the complexation of β-CD with eight other substituted benzenes were determined with the new method.

Results and discussion

For MHB, UV measurement was conducted at the wavelength of 255.5 nm. ϵ_0 was 1.48×10^3 m^2/mol, and $\Delta\epsilon = \epsilon_\infty - \epsilon_0 = -159$ m^2/mol. The BH method gave an association constant of 710 M^{-1} with a correlation coefficient of 0.986. The new method gave an association constant of 693 M^{-1}. Considering a reasonable relative error of 0.3% from preparing solutions and a reasonable relative error of 0.2% from measuring the UV absorption [20], a reasonable criterion to judge the experiment with the new method was proposed that any *rsd* in the experiment should be less than 0.5%. Herein, the largest *rsd* was 0.4%.

As seen, the association constants by the two methods were similar, although obviously the new method used less data and thus required less labor. Hence, the new method is reliable and convenient for measuring the association constants.

The association constants for the complexation of β-CD with eight other substituted benzenes are listed in Table 1. As seen, the results are in agreement with the values in the literature. This further indicated that the new method was reliable.

Computer simulations

Simulation method

For better comparison of the BH method with the new one, computer simulation was performed. The computer programs, written in Borland C++ 5.0, were run on a PII400 computer.

In the simulation, the expected concentration of the substrate was chosen as 1.00×10^{-4} M for all the solutions. The expected associations constant (K) was chosen as 50, 1000 or 1000 M^{-1}. The molar absorption constant of the free substrate (ϵ_0) was taken as 5000 m^2/mol, while the molar absorption constant of the bound substrate (ϵ_∞) was chosen as 4950, 4850, or 4750 m^2/mol.

Table 2. The optimum value for C^1_{CD} in the computer simulation

C^1_{CD}	K = 50 M^{-1}	K = 1000 M^{-1}	K = 10000 M^{-1}
$\Delta\epsilon = -50$ m^2/mol	2.4×10^{-3} M	0.9×10^{-3} M	0.2×10^{-3} M
$\Delta\epsilon = -150$ m^2/mol	3.8×10^{-3} M	1.0×10^{-3} M	0.2×10^{-3} M
$\Delta\epsilon = -250$ m^2/mol	4.2×10^{-3} M	1.0×10^{-3} M	0.2×10^{-3} M

For the BH method, two cases were considered. In one case, five data points were used, and the expected host concentrations were chosen as 0.0000, 0.0025, 0.0050, 0.0075 and 0.0100 M, respectively. In the other case, seven data points were used, and the expected host concentrations were chosen as 0.0000, 0.0025, 0.0040, 0.0055, 0.0070, 0.0085 and 0.0100 M, respectively. Three replicate solutions were prepared at every host concentration, and their absorption values were averaged to offer the absorption value at the given host concentration.

In the new method, four replicate solutions were prepared at every host concentration, and their absorption values were averaged to offer the absorption value at the given host concentration. C^2_{CD} was held constant at 1.00×10^{-2} M, while C^1_{CD} was changed from 1.0×10^{-3} to 3.0×10^{-3} M by a step of 0.1×10^{-3} M. The value that yielded the minimum error in measuring the association constant was chosen as the experimental C^1_{CD} (see Table 2).

Only the random errors from preparing the solutions and from measuring the absorption values were considered in the simulation. Systematic errors, which could and should be avoided, were assumed to be zero. Thus, the final absorption values taken by the computer to calculate the association constants were not determined merely by the expected concentrations of the solutions but by the influence of the random errors as well. The random error from preparing the solutions was assumed to obey the normal error curve model, i.e.,

$$y = \frac{1}{\sigma\sqrt{2\pi}} e^{-((x-\mu)^2/2\sigma^2)}. \quad (7)$$

Here, μ was the expected concentration of the given solution, while x was the concentration actually prepared. σ was the standard deviation, which was chosen as 0.003μ here. y was the relative frequency with which random sampling of the infinite population would bring about a particular concentration x. Similarly, the random error from measuring the absorption value was also assumed to obey the normal error model, wherein σ was chosen as 0.002μ.

One million rounds of simulations were performed for every case (see Chart 1). In every round, when the computer generated the final absorption values of the solutions, a test was performed. For the BH method, the test was whether the correlation coefficient (r) of regression was larger than a criterion. (Here the criterion was chosen as $r > 0.98$) If it was, the round of simulation corresponded to a successful experiment. For the new method, the test was whether any *rsd* in this round of simulation was smaller than a criterion. (Here the criterion was chosen as $rsd < 0.5\%$) After one million rounds, the relative error in the association constants ($\delta K/K$) and the success rate (the ratio of the number of successful round (N) to one million) were obtained.

Chart 1

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Simulation Algorithm ( )
{   Summation_δK = 0; N = 0;
    For i = 1 to 1000000
      {   Generate the absorption values of the solutions;
          Calculate r (or rsd) using the present absorption values;
          If r > 0.98 (or rsd< 0.5%
          {   Calculate K_i according to the BH (or the new) method;
              Summation_δK = abs(K_i − K); N = N + 1;
          }
      }
    δK/K = (Summation_δK/N)/K; Success_Rate = N/1000000;
}
```

Results and discussion

The accuracy in measuring the association constants

Table 3 illustrates the relative errors and success rates in measuring the association constants with the BH and with the new method. As seen, the relative error by the BH method was generally large (mostly over 30%). This explained why the difference between the association constants reported in the literature for the same substrate was often so great, although the reported correlation coefficients were usually excellent [32, 38]. Interestingly, the seven-point BH method yielded only slightly more accurate results than the five-point one. Thus, increasing the datum points might not effectively increase the accuracy. Although the BH method was acceptably accurate when K = 1000 M^{-1}, it tended to give poor estimation when K = 10000 M^{-1}or K = 50 M^{-1}. This explained why an accurate association constant was often difficult to obtain with the BH method when the complexation was very weak or very strong [40].

In comparison, the new method had a significantly smaller relative error than the BH method in measuring the association constants. An only exception was when K = 50 M^{-1} and $\Delta\epsilon$ = −50 m^2/mol. As seen, when the complexation was either modest or strong, the new method could always provide satisfactory estimations.

Table 3. The relative error and success rate in measuring the association constants with the five-point BH method, the seven-point BH method, and the new method

K (M^{-1})	ϵ (m^2/mol)		Five-point BH	Seven-point BH	New method
50	−50	*Relative error*	1.570	1.294	2.560
		Success rate	0.280	0.070	0.640
	−150	*Relative error*	0.816	0.694	0.534
		Success rate	0.890	0.680	0.910
	−250	*Relative error*	0.534	0.490	0.326
		Success rate	0.990	0.940	0.950
1000	−50	*Relative error*	0.332	0.339	0.237
		Success rate	0.230	0.050	0.950
	−150	*Relative error*	0.120	0.111	0.074
		Success rate	0.890	0.710	0.950
	−250	*Relative error*	0.074	0.069	0.043
		Success rate	1.000	0.990	0.950
10000	−50	*Relative error*	0.851	0.853	0.238
		Success rate	0.030	0.003	0.950
	−150	*Relative error*	0.458	0.472	0.072
		Success rate	0.060	0.005	0.950
	−250	*Relative error*	0.298	0.315	0.042
		Success rate	0.140	0.020	0.940

The superiority of the new method over the BH method indicated that the latter did not efficiently handle the experimental data. In the BH method, the reciprocals of the concentration and absorption values were used. Since a small value had a large reciprocal, the slope of regression was only sensitive to the points with small concentrations [12]. The points with large concentrations played unimportant roles in regression, although their presence required much labor and time.

The success rate of the measurement

From Table 3, the success rates of the BH method were generally not high, in agreement with the experimental experience that a high correlation coefficient was often hard to obtain in the BH method. Since any result with a low correlation coefficient was usually deemed questionable, it turned out that experiments using the BH method would often be unsatisfactory. The seven-point BH method had an even lower success rate than the five-point one. When $\Delta\epsilon$ = −50 m^2/mol or when K = 10000 M^{-1}, the success rate was extremely low. Hence, the BH method was not recommendable if the complexation was strong or if the absorption change was small.

In comparison, the success rates of the new method were mostly around 95%, regardless of the strength of complexation and extent of absorption change. Thus experiments using the new method should usually be successful if properly conducted. This was understandable, for the selection

of criterion in the new method had adequately considered the possible sources of the random errors.

Summary remarks

In brief, the relative error of the new method is generally lower than that of the BH method, while the success rate of the former is generally higher than that of the latter. It seems that the BH method is only recommended when the complexation is modest (i.e., $K \approx 1000\ \mathrm{M}^{-1}$) and the absorption change is not small (i.e., $|\Delta\epsilon| > 100\ \mathrm{m}^2/\mathrm{mol}$). In comparison, the new method is generally applicable, except when the complexation is very weak and the absorption change is very small.

In addition, the requirement that $C_{CD} \gg C_S$ in the BH method is often inapplicable, and in consequence sometimes the association constants cannot be measured with the BH method [41]. However, the new method does not have such a requirement; thus, it is more widely applicable.

Admittedly, the new method has an unavoidable shortcoming, for it is based on the assumption that only the 1:1 complex is formed. Although this approximates most of the cases in practice, some outliers can be occasionally encountered. Hence, caution should be given in applying the new method to a new host–guest complex. However, it is noteworthy that the BH method was also found questionable in dealing with the same problem [42]. Nevertheless, for most known host–guest complexation, the new method is safely applicable.

Conclusion

A new method is proposed for measuring association constants. Experimental practice shows that the new method is reliable and convenient. Computer simulation demonstrates that the new method has a significantly improved accuracy and efficiency compared with the BH method. Hence, the new method is a novel recommendable approach for a convenient and reliable measurement of association constants.

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