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CONSTEQ – A PROGRAM FOR THE CALCULATION OF THE EQUILIBRIUM CONSTANTS USING SPECTROSCOPIC DATA

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ABSTRACT. When working in the field of host-guest supramolecular chemistry in order to quantitatively appreciate the complex formation, the association constants have to be determined. For this purpose, in this contribution, a non-linear least square curve-fitting program, ConstEq, developed in our group, is presented.

1. Introduction

The formation of a complex between a host and a guest molecule is a basic and important process in supramolecular chemistry. In this field the association constant (K_a) determination represent an important part of research because this is the factor which quantitatively characterize the complex formation [1-2]. During the time, a wide variety of methods for the determination of stability constants have been developed based on potentiometric, spectrophotometric and NMR data. They can be classified in two categories: graphical (or linearization) methods and curve-fitting non-linear methods. If graphical methods are designed to produce a linear relationship between the experimental data and K_a, some approximations have to be made. The curve-fitting methods require no approximation and allow an almost unrestricted distribution of experimental points (concentrations). The curve-fitting methods are correct data treatments and will produce the most reliable and accurate measurements of K_a. Consteq represents our group contribution in this field.

2. Theory

The theoretical analysis of a host-guest complexation is based on a simple equilibrium model in solution:

$$a \cdot H + b \cdot G \leftrightarrow H_a G_b = C\,. \tag{1}$$

The association constant is defined by the following equations:

$$K = \frac{[C]}{[H]^a [G]^b} \tag{2}$$

$$[H]_t = [H] + a \cdot [C] \tag{3}$$

$$[G]_t = [G] + b \cdot [C], \tag{4}$$

where H is the host, G the guest, C the complex; *a*, *b*, the stoichiometry; $[H]_t$ and $[G]_t$ are the total concentration of the host (guest) molecules at initial state; $[H]$, $[G]$ and $[C]$ represents the host, guest and complex concentrations at final stage, namely at equilibrium. Using the equations (3)-(4) we can derive:

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

The first step in order to determine the binding constant is the determination of stoichiometry, namely *a* and *b*. The most popular method for doing this is the Continuous Variation Method [3], using a Job plot. Even if practically the concentration of the complex, $[C]$, could not be measured directly, it can be replaced with a parameter proportional to $[C]$. For example, when the complex 1:1 is predominant at equilibrium, the maximum in the Job's plot appears at $x = 0.5 (a = b = 1)$. In the case of 1:2 complexation the maximum is at $x = 0.333$. Depending on each experiment, there is a spectral parameter suitable for the replacement of $[C]$. For NMR spectroscopy the parameter is the chemical shift δ, and for UV-visible spectroscopy is the absorbance, A.

In the following, as the chemical shift parameter, δ, in an NMR experiment, is most sensible at the modification of the chemical environment, we will particularize for this case. Also, we consider here only the case of a fast chemical exchange, on the NMR scale, of the guest molecule G between the complexed and free state. In this case, the observed chemical shift, δ_{obs}, is the weight average of the chemical shifts corresponding to the free state, δ_f, and to the pure complexed state, δ_c, and we have:

$$\delta_{obs} = \frac{[G]\delta_f + b[C]\delta_c}{[G]_t} \tag{6}$$

which can also be written:

$$\delta_{obs} = z \cdot \delta_f + \delta_c \cdot (1 - z) \quad where \quad z = \frac{[G]}{[G]_t}. \tag{7}$$

In this equation we observe that when $[G] \approx 0$, meaning that practically all guest molecules are complexed, $z \approx 0$ and $\delta_{obs} \to \delta_c$. When $[G] = [G]_t$ or $[G]_t >> [H]_t$, $z \approx 1$ and $\delta_{obs} \to \delta_f$. For the chemical shifts of the host molecule similar equations can be derived. If we note with $\Delta\delta_{obs} = \delta_f - \delta_{obs}$, the observed difference in the chemical shift and with $\Delta\delta_c = \delta_f - \delta_c$, the chemical shift difference (for a given proton) between the free component and the pure inclusion complex, we can derive the expression:

$$\Delta\delta_{obs}^{(X)} = \frac{[C]}{[X]_t} \cdot \Delta\delta_c^{(X)}, \qquad (8)$$

where $X = H$ or G. For a 1:1 complex ($a = b = 1$), the substitution of the expression (8) in eq. (5) leads us to the following equation:

$$[X]_t^2 \left(\Delta\delta_{obs}^{(X)}\right)^2 - [X]_t \Delta\delta_{obs}^{(X)} \Delta\delta_c^{(X)} \left\{[M] + \frac{1}{K}\right\} + [H]_t [G]_t \left(\Delta\delta_c^{(X)}\right)^2 = 0, \qquad (9)$$

where $[M] = [H]_t + [G]_t$.

The solution of this equation is:

$$\Delta\delta_{obs}^{(X)} = \frac{\Delta\delta_c^{(X)}}{2[X]_t} \times \left\{[M] + \frac{1}{K} \pm \left[\left([M] + \frac{1}{K}\right)^2 - 4[H]_t [G]_t\right]^{1/2}\right\}, \qquad (10)$$

and only the "-" solution has a physical meaning because the ratio $\Delta\delta_{obs}/\Delta\delta_c$ must always be lower than 1.

The principle of non-linear curve fitting methods is that with knowledge of the complex stoichiometry a binding isotherm can be calculated (e.g. using eq. (10)) and compared with the experimental data. $\Delta\delta_c$ and the association constant K are separate variables and the correct values of $\Delta\delta_c$ and K are those that produce the best fit of calculated to observed data $\Delta\delta_{obs}$.

3. Description of CONSTEQ

As we have seen above, the equation (10) correlates, in the case of a complex with the stoichiometry 1:1, the total concentration of the guest and host molecules with the observed difference in chemical shift $\Delta\delta_{obs}$ and involves no approximation. Our computer program 'CONSTEQ' adjusts the parameters (K and $\Delta\delta_c$) in the equation (10) to obtain the best fit to the experimental values $\Delta\delta_{obs}$. Each iteration sets up a quadratic procedure to determine the direction of search and calculates the error function:

$$E = \sum_{i,j} \left(\Delta\delta^{(i,j)} - \Delta\delta_{calc}^{(i,j)}\right)^2, \qquad (11)$$

where *i* counts the sample number and *j* the studied proton. The fitting procedure reaches the end when the difference between two consecutive E values is smaller than 10^{-6}. The treatment of the whole set of protons produces one single K value using the NMR chemical shift variations as a function of guest or host concentrations. The program is quite flexible since up to a total of 15 guest and host NMR lines can be used in the fitting procedure. It provides also the chemical shift values of the NMR lines in the pure complex.

4. Conclusions

CONSTEQ is written entirely in C++, consists of more than 1300 lines of code, is distributed as an executable file and can be obtained freely on demand. It runs under Windows 95/98/XP, even if practically is a DOS program. In the future we will consider the development of a Windows interface and also the calculation of stability constants for complexes with 1:2 or 2:1 stoichiometry. The program was successfully used in a series of papers concerning the drug cyclodextrin inclusion process [4-6].

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