USER FUNCTION IN THE CURVE-FITTING PROCESS FOR DETERMINATION OF THE MULTIPLE EQUILIBRIA BINDING CONSTANTS

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abstract: User function that can be used in the curve fitting for the determination of the binding constants in the case of multiple equilibria was defined. This function, written in C language, is based on the exactly solution of the cubic equation obtained when both 1:1 and 1:2 complexes are simultaneously present in the system.

Introduction

The problem of multiple equilibria consisting in the formation of two or more complexes has been dealt with in several fields. Thus, acid-base equilibria, metal ion-ligand coordination, as well as binding of small molecules to macromolecules including biopolymers, like enzymes and nucleic acids, and synthetic polymers, require consideration of multiple binding.

Even in the field of small molecule-small molecule organic complexes, careful investigation sometimes reveals that it is necessary to take account of additional complex species [1÷5].

In many cases, the determination of the stepwise binding constants is very difficultly, if not impossible, to do, the experimental property measured being a sum of the contribution of all components present in the system. Various graphical method are used consisting in some equation linearization, linear extrapolations, Taylor's series expansion and linear approximation [6,7]. The most serious disadvantage of these methods is that the stability constants of complicated systems can be evaluated only in several steps when an accumulation of errors occurs.

The best way to estimate the binding constants for multiple equilibria is nonlinear regression analysis.

In this paper it is developed a general method for the determination of the stepwise binding constants, when both 1:1 and 1:2 complex formation is taken into account. This method uses as curve-fitting function a cubic equation obtained for this systems writing the

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stepwise constants K_{11} and K_{12} as a function of the total transformation degree of the ligand into 1:1 and 1:2 complexes.

Theoretical

When both 1:1 and 1:2 complexes (SL and SL$_2$) are present in the medium, it is chemical reasonable to assume that every complex is formed in a bimolecular process, so these two complexes are related by following equilibria:

$$S + L \rightleftharpoons SL \tag{1}$$

$$SL + L \rightleftharpoons SL_2 \tag{2}$$

and the stepwise binding constants K_{11} and K_{12} are defined by:

$$K_{11} = \frac{[SL]}{[S][L]} \tag{3}$$

$$K_{12} = \frac{[SL_2]}{[SL][L]} \tag{4}$$

Any observable property of the system can be written as a function on the degree of the transformation of the ligand into both complexes (α), which is a sum of the ligand fractions in the form of species SL (α_{11}) and SL$_2$ (α_{12}). The following relations define these fractions:

$$\alpha_{11} = \frac{[SL]}{L_t} \text{ and } \alpha_{12} = \frac{[SL_2]}{L_t} \tag{5}$$

$$\alpha = \alpha_{11} + \alpha_{12} \tag{6}$$

The mass balance expressions for S and L are:

$$S_t = [S] + [SL] + [SL_2] = [S] + \alpha_{11}L_t + \frac{\alpha_{12}}{2}L_t \tag{7}$$

$$L_t = [L] + [SL] + 2 \cdot [SL_2] = [L] + \alpha_{11}L_t + \alpha_{12}L_t \tag{8}$$

In equations (5)-(8) S_t and [S] represent total and free (unbound) substrate concentrations, respectively, and similarly for the ligand.

From (7) and (8) the concentrations of free substrate and free ligand can be written as a function of the ratio of initial concentrations of the substrate and ligand $X = S_t/L_t$.

After the expansion of the binding constants K_{11} and K_{12} in terms of α_{11}, α_{12} and α, equation (6) leads to the following third order equation in α:

$$\alpha^3 - [(A + 2(1 + X)] \cdot \alpha^2 + [1 + (4 + A)X + A(1 + B)] \cdot \alpha - (2 + A)X = 0 \tag{9}$$

where X is the ratio of total concentrations of the substrate and ligand $X = S_t/L_t$.

The parameters A and B from the equation (9) are correlated with stepwise binding constants by relation (10):

$$A = \frac{1}{K_{12} \cdot L_t} \quad \text{and} \quad B = \frac{1}{K_{11} \cdot L_t} \tag{10}$$

the concentration of the ligand Lt being constant

These parameters will became the fitting parameters in the user function together with the ones that determine the analytical response (absorbance, chemical shift, conductivity, etc.)

One of the real solutions of the equation (9) has the physical significance of the total degree of ligand transformation. The nature of the roots is determined by the value of the dicriminant of the equation [8].

The first part of the user function needed in the fitting process is dedicated to solution of the equation (9). The results of this part, α_{11} and α_{12}, are used in the second part to compute analytical response of the system as a function of the ligand fractions in the form of species SL (α_{11}), respectively SL$_2$ (α_{12}).

User function is a part of a little program written in C language which can be easily adjusted (and translated) to any iterative fitting program based on the least squares method. This user function is:

```
q=1/3*(2+A+2*X)*(1+A*(1+B)+(4+A)*X)-2/27*(2+A+2*X)
*(2+A+2*X) *(2+A+2*X)-(2+A)*X;

p=1+A*(1+B)+(4+A)*X-1.0/3*(2+A+2*X)*(2+A+2*X);

delta=q/2*(q/2)+p/3*(p/3)*(p/3);

if (delta > 0)
{
f1 = -q/2 + sqrt(delta);
f2 = -q/2 - sqrt(delta);
if (f1 < 0) f3 = -exp(1/3 * log(fabs(f1)));
else f3 = exp(1/3 * log(fabs(f1)));

if (f2 < 0) f4 = -exp(1/3 * log(fabs(f2)));
else f4 = exp(1/3 * log(fabs(f2)));

alfa = f3 + f4 + 1/3 * (2 + A + 2*X);
}
else
{
f1 = sqrt(fabs(p/3*(p/3)*(p/3)));
cosfi = -q/f1 /2;
f2=acos(cosfi);
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 XX = exp(1.0 / 3 * log(fabs(f1)));

 alfa = 2*XX*cos(f2/3+ 2/3*M_PI) + 1/3*(2 + A + 2 * X);
     }
 alfa11 = (1 - alfa) * (2*X - alfa) / (2*B + 1 - alfa);
 alfa12 = alfa -alfa11;
```

property = C * alfa11 + D * alfa12;

Discussion

The last line of the function computes the **property** of the system, *C* and *D* being another two fitting parameters that correlated the transformation degrees of the ligand with this property.

For example, when experimental method is optical spectroscopy, the property is the change in the absorbance of the system ΔA, and a wavelength is selected so that the molar absorptivities (ε_i) of the present species are different:

$$\Delta A = (\Delta\varepsilon_{11}\alpha_{11} + \Delta\varepsilon_{12}\alpha_{12})\cdot L_t \qquad (11)$$

where $\Delta\varepsilon_{11} = \varepsilon_{SL} - \varepsilon_S - \varepsilon_L$ and $\Delta\varepsilon_{12} = \varepsilon_{SL_2} - \varepsilon_S - \varepsilon_L$.

In this case the last line of the function has to be written:

$$\textbf{property} = (C * alfa11 + D * alfa12)*Lt ; \qquad (12)$$

if the concentration of the ligand L_t is held fixed; *C* and *D* represent the changes in the molar absorptivities $\Delta\varepsilon_{11}$ and $\Delta\varepsilon_{12}$.

If the experimental data consist of recorded values of the **property** by varying the ligand concentration at constant total substrate concentration, a substitution has to be made. Thus the parameters A and B become:

$$A = \frac{1}{K_{12}\cdot S_t}\cdot X \text{ and } B = \frac{1}{K_{11}\cdot S_t}\cdot X \qquad (13)$$

and now *the new fitting parameters* will be:

$$AA = \frac{1}{K_{12}\cdot S_t} \text{ and } BB = \frac{1}{K_{11}\cdot S_t} \qquad (14)$$

As a result of this substitution the parameters A and B have to be replace in equation (9) as well as in the user function, with *AA**X and *BB**X. The last line becomes:

$$\textbf{property} = (C * alfa11 + D * alfa12)*St/X ; \qquad (15)$$

Both equation (9) and user function present flexibility and can be modified by adequate substitution if it is required by the experimental reasons.

The user function was tested for some hypothetical systems with binding constants K_{11} and K_{12} varying on a large range. There is an ambiguity into the results of the fitting process only for the low value for K_{11} or K_{12}. In these cases only a statistical analyses can discriminate between a false or a true result and, in general, is necessary to repeat the fitting process using different initial estimates of the parameters till all of them is obtained with an acceptable standard error.

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