

Exchange Algorithm for Origin® 7.0

Analysis of the Exchange Reaction $AB + CD \rightleftharpoons AC + BD$ by Isothermal Titration Calorimetry

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Installation

The algorithm is developed for Origin® version 7.0 taking advantage of the Origin® C compiler.

Running the Exchange routine for the first time

- 1) Rename the Origin® Function Definition file *Exchange.txt* to *Exchange.fdf*. Copy *Exchange.fdf* to the \Fitfunc subdirectory under the Origin® directory.
- 2) Open Origin® for ITC and read in an ITC dataset. Perform the usual integrations, baseline corrections, etc.
Alternatively, open a saved *.DH file with peak areas or an Origin® Project File (*.OPJ).
- 3) Click Math.
Click Select Fitting Function...
- 4) If not already selected, select ITC in Categories.

The first time this routine is used, *Exchange* will not be in the Function list.

Click Function.

Click Add.

Find the \Fitfunc subdirectory under Origin®.

Click *Exchange.fdf*.

Click Open.

Continue with item 5) below (*Running the Exchange routine*).

Running the Exchange routine

- 1) Open Origin for ITC and read in an ITC dataset. Perform the usual integrations, baseline corrections, etc.
Alternatively, open a saved *.DH file with peak areas or an Origin® Project File (*.OPJ).
- 2) Click Math.
Click Select Fitting Function...
- 3) If not already selected, select ITC in Categories.

- 4) Select the Exchange function
- 5) Click Action.
Click Dataset.
The NDH variable is highlighted. Click the corresponding dataset (*_NDH) in Available Datasets.
Click Assign. This assigns NDH (heat signals) as the dependent variable.
Click --->XMt in Variables.
Click the corresponding dataset (*_XMt) in Available Datasets.
Click Assign. This assigns XMt (mole ratio) as the independent variable.
Click --->injV in Variables.
Click the corresponding dataset (*_injV) in Available Datasets.
Click Assign. This assigns injV (injection volume) as another independent variable.

NOTE! It is important that the last two assignments are carried out in this specific order. If not, the fitting will be OK, but Origin® will plot the results with injection volumes as x-axis.

- 6) Click Action.
Click Fit.
Enter the initial values of the fitting parameters:

N is the stoichiometry.*

K is the equilibrium constant of the reaction (dimensionless).

DH is the corresponding change in standard reaction enthalpy in cal mol^{-1} .

AB0 is the initial concentration of species AB in the sample cell in mM.

CDsyr is the concentration of species CD in the syringe in mM.

CD0 is the initial concentration of species CD in the sample cell in mM (default: 0 mM).

BD0 is the initial concentration of species BD in the sample cell in mM (default: 0 mM).

hd is the heat of dilution of species CD in cal mol^{-1} .

V0 is the sample cell volume in mL.

- 7) Click one of the Iter. buttons to perform the fit.

*The stoichiometry parameter N is a "correction parameter" that can be used if either A0 or C0syr (but not both) is not known precisely, or if the stoichiometry differs from 1:1.

Theory

The routine is for analysis of an equilibrium represented by the general scheme:



The equilibrium constant for this reaction is

$$K = \frac{[\text{AC}][\text{BD}]}{[\text{AB}][\text{CD}]}. \quad (2)$$

The following mass preservation equations can be written:

$$[A]_{\text{Total}} = [AB] + [AC], \quad (3)$$

$$[B]_{\text{Total}} = [AB] + [BD], \quad (4)$$

$$[C]_{\text{Total}} = [AC] + [CD], \quad (5)$$

and

$$[D]_{\text{Total}} = [BD] + [CD]. \quad (6)$$

It is assumed that the only species containing the A moiety in the calorimetric cell before the first injection is AB and that CD is the only species in the titration syringe. There may then be up to three of the reactants in the calorimetric cell at the beginning: AB, BD, and CD. The initial concentrations of all three must be known. In most experiments the initial concentrations of BD and CD would be zero. Now, we have

$$[A]_0 = [AB]_0 \quad (7)$$

and

$$[B]_0 = [AB]_0 + [BD]_0 = [A]_0 + [BD]_0. \quad (8)$$

The total (stoichiometric) concentrations of moieties A, B, and C after each injection are given by the following relations taking dilution and liquid displacement into account (I):

$$[A]_{\text{Total}} = [A]_0 \exp\left(-\frac{1}{V_{\text{cell}}} \sum_{j=1}^i V_j\right), \quad (9)$$

$$[B]_{\text{Total}} = [B]_0 \exp\left(-\frac{1}{V_{\text{cell}}} \sum_{j=1}^i V_j\right), \quad (10)$$

and

$$[C]_{\text{Total}} = [CD]_{\text{Syringe}} \left\{1 - \exp\left(-\frac{1}{V_{\text{cell}}} \sum_{j=1}^i V_j\right)\right\} + [CD]_0 \exp\left(-\frac{1}{V_{\text{cell}}} \sum_{j=1}^i V_j\right), \quad (11)$$

where $[CD]_{\text{Syringe}}$ is the concentration of CD in the syringe, $[CD]_0$ is the stoichiometric concentration of CD before the initiation of the titration, and V_j is the injection volume of the j 'th injection. Substitution of Equations (3)-(5) and (7)-(11) into Equation (2) gives

$$K = \frac{[AC]([B]_{\text{Total}} - [A]_{\text{Total}} + [AC])}{([A]_{\text{Total}} - [AC])([C]_{\text{Total}} - [AC])}, \quad (12)$$

and this quadratic equation may be solved with respect to $[AC]$:

$$[AC] = \frac{K}{2(K-1)} \left([A]_{\text{Total}} + [C]_{\text{Total}} + \frac{[A]_{\text{Total}} - [B]_{\text{Total}}}{K} \right) \pm \sqrt{\left([A]_{\text{Total}} + [C]_{\text{Total}} + \frac{[A]_{\text{Total}} - [B]_{\text{Total}}}{K} \right)^2 - \frac{4(K-1)[A]_{\text{Total}}[C]_{\text{Total}}}{K}}. \quad (13)$$

In Equation (13) the plus sign in front of the square root pertains to $K < 1$ and the minus sign to $K > 1$. The sign can be determined by a parameter, s , defined as

$$s = \frac{K-1}{|K-1|} \quad (K \neq 1), \quad (14)$$

which is $s = 1$ for $K > 1$ and $s = -1$ for $K < 1$. Equation (13) can then be written as

$$[AC] = \frac{K}{2(K-1)} \left([A]_{\text{Total}} + [C]_{\text{Total}} + \frac{[A]_{\text{Total}} - [B]_{\text{Total}}}{K} \right) - s \sqrt{\left([A]_{\text{Total}} + [C]_{\text{Total}} + \frac{[A]_{\text{Total}} - [B]_{\text{Total}}}{K} \right)^2 - \frac{4(K-1)[A]_{\text{Total}}[C]_{\text{Total}}}{K}}. \quad (15)$$

For the trivial case in which $K = 1$, one obtains from Equation (12):

$$[AC] = \frac{[A]_{\text{tot}}[C]_{\text{tot}}}{[B]_{\text{tot}} + [C]_{\text{tot}}} \quad (16)$$

Most often the products will be present in equimolar concentrations: $[AC] = [BD]$. In these cases $[A]_{\text{Total}} = [B]_{\text{Total}}$ and Equation (15) reduces to

$$[AC] = \frac{K}{2(K-1)} \left([A]_{\text{Total}} + [C]_{\text{Total}} - s \sqrt{([A]_{\text{Total}} + [C]_{\text{Total}})^2 - \frac{4(K-1)[A]_{\text{Total}}[C]_{\text{Total}}}{K}} \right). \quad (17)$$

The heat change, q_i , after the i 'th injection is given by

$$q_i = V_{\text{cell}} \Delta H^\circ \Delta[AC]_i, \quad (18)$$

where ΔH° is the standard molar enthalpy change of the reaction and $\Delta[AC]_i$ is the change in the concentration of AC ($= \Delta[BD]_i$), which is given by

$$\Delta[AC]_i = [AC]_i - [AC]_{i-1} \exp\left(-\frac{V_i}{V_{\text{cell}}}\right). \quad (19)$$

The exponential expression in Equation (19) is the dilution factor of the i 'th injection.

The apparent equilibrium constant, K , and ΔH° are determined from the ITC isotherms by nonlinear least-squares regression analysis using Equations (7)-(11), (15), (17), and (19). The standard free energy change, ΔG° , and standard entropy change, ΔS° , are calculated from the standard thermodynamic relations:

$$\Delta G^\circ = -RT \ln K = \Delta H^\circ - T\Delta S^\circ. \quad (20)$$

The degree of reaction can be defined as

$$\phi = \frac{[AC]}{[A]_{\text{Total}}} \quad (21)$$

and the molar ratio as

$$r = \frac{[C]_{\text{Total}}}{[A]_{\text{Total}}}. \quad (22)$$

Dividing Equation (15) by $[A]_{\text{Total}}$ on both sides, substitution with Equations (21) and (22), and assuming that $[A]_{\text{Total}} = [B]_{\text{Total}}$ yields

$$\phi = \frac{K}{2(K-1)} \left(1 + r - s \sqrt{(1+r)^2 - \frac{4(K-1)r}{K}} \right). \quad (23)$$

Differentiation of this equation with respect to r gives

$$\frac{d\phi}{dr} = \frac{K}{2(K-1)} \left(1 - s \frac{1 + r - \frac{2(K-1)}{K}}{\sqrt{(1+r)^2 - \frac{4(K-1)r}{K}}} \right). \quad (24)$$

Equation (24) describes the ITC isotherm and this must have a sigmoid shape to obtain reliable values from fitting. This corresponds to the c -value for the *OneSites* model (2). Plots of $d\phi/dr$ versus the molar ratio (r) for different values of K and for $s = 1$ according to Equation (24) are shown in the figure below. It is clear from this figure that sigmoid curves are obtained for $5 < K < 10^4$, and this is the range in which reliable values of K may be obtained. For values of K smaller than 0.2 and down to 10^{-4} the titration should be reversed.

References

1. Bundle, D. R. and Sigurskjold, B. W. (1994) Determination of Accurate Thermodynamics of Binding by Titration Microcalorimetry, *Methods Enzymol.* 247, 288-305.
2. Wiseman, T., Williston, S., Brandts, J. F., and Lin, L.-N. (1989) Rapid Measurement of Binding Constants and Heats of Binding Using a New Titration Calorimeter, *Anal. Biochem.* 179, 131-137.

