

ON THE DETERMINATION OF REACTION VOLUMES FOR COUPLED EQUILIBRIA

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The volume change of a chemical reaction is usually determined by measurements of the equilibrium constant at different pressures. This can be a tedious process for coupled equilibria, requiring a large amount of data over a wide pressure range. In this communication we suggest a procedure for determining reaction volumes which has certain advantages over the standard approach. The method consists of determining the equilibrium constants of a system at a single pressure, usually atmospheric pressure, and then measuring the signal change (light absorbance, conductivity, etc.) as a function of reactant concentrations for a small perturbation from the initial pressure. The small perturbation condition allows use of linear equations which can be derived by simple inspection of the reaction scheme and solved with linear regression analysis. This method can be used for the quantitative analysis of overall amplitudes observed in pressure-jump relaxation experiments.

It can be shown (1-3) that for small pressure-induced perturbations of equilibrium concentrations, the change in absorbance of a reaction solution is in general given by the following relation<sup>a,b</sup>

$$\Delta A^* = b_{11}X_{11} + b_{12}X_{12} + \dots + b_{1R}X_{1R} \\ + b_{22}X_{22} + \dots + b_{2R}X_{2R} \\ \dots \\ + b_{RR}X_{RR} \quad (1)$$

where  $R$  is the number of reaction steps, the  $b_{\alpha\beta}$  are related in a simple way to elementary volume changes and extinction coefficient changes of elementary steps,

$$b_{\alpha\alpha} = \Delta\epsilon_{\alpha} \Delta V_{\alpha} \quad (2)$$

$$b_{\alpha\beta} = \Delta\epsilon_{\alpha} \Delta V_{\beta} + \Delta\epsilon_{\beta} \Delta V_{\alpha} \quad (3)$$

and the  $X_{ij}$  are functions of stoichiometric coefficients and equilibrium concentrations at atmospheric pressure.

Expressions for the  $X_{ij}$  of eq. 1 are easily derived by the following procedure:

1. Number the reaction steps 1, 2 ... R, and the chemical components 1, 2 ... N.
2. Construct an  $R \times R$  symmetrical matrix  $\underline{g}$  whose elements are given by

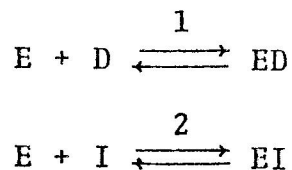
$$g_{\alpha\beta} = \sum_{i=1}^N \frac{v_{i\alpha} v_{i\beta}}{\bar{C}_i} \quad (4)$$

where  $V_{i\alpha}$  is the stoichiometric coefficient of species  $i$  in reaction  $\alpha$  (coefficients are assumed positive for products and negative for reactants), and  $\bar{C}_i$  is the molar equilibrium concentration of species  $i$  at atmospheric pressure.

3. Evaluate the inverse matrix  $g^{-1}$ . The elements of  $g^{-1}$  are the  $X_{ij}$  of eq. 1.

We may consider eq. 1 a linear regression equation in which the experimental  $\Delta A^*$  is the dependent variable, the  $X_{ij}$  are independent variables and the  $b_{\alpha\beta}$  are regression coefficients which can be determined with a least-squares analysis. The reaction volumes are then calculated from the  $b_{\alpha\beta}$  and the extinction coefficient changes using eqs. 2 and 3. It is assumed that the extinction coefficient differences are independent of pressure.

As an illustration of the method we shall consider the competitive binding of two inhibitors for the active site of an enzyme:



In the general case where no restrictions are placed on extinction coefficient changes or reaction volumes, a pressure-induced shift in equilibrium concentrations will result in an absorbance change described by the following relation,

$$\Delta A^* = b_{11}X_{11} + b_{12}X_{12} + b_{22}X_{22} \quad (5)$$

where the constant terms are

$$\begin{aligned}
 b_{11} &= \Delta\varepsilon_1 \Delta V_1, \quad b_{22} = \Delta\varepsilon_2 \Delta V_2 \\
 b_{12} &= \Delta\varepsilon_1 \Delta V_2 + \Delta\varepsilon_2 \Delta V_1
 \end{aligned}
 \tag{6}$$

and the  $X_{ij}$  are the elements of  $\underline{g}^{-1}$ , where

$$\begin{aligned}
 g_{11} &= 1/\bar{E} + 1/\bar{D} + 1/\bar{ED} \\
 g_{22} &= 1/\bar{E} + 1/\bar{I} + 1/\bar{EI} \\
 g_{12} &= 1/\bar{E}
 \end{aligned}
 \tag{7}$$

In the limiting case where only D and ED absorb significantly at the chosen wavelength, the binding of I to E does not in itself give rise to a signal change. Nevertheless, due to coupling between the two equilibria, the pressure-induced absorbance change is still a function of  $\Delta V_2$ , as seen by setting  $\Delta E_2 = 0$  in eq. 5. The final result is

$$\begin{aligned}
 \Delta A^* &= \frac{[\bar{E}^{-1} + \bar{I}^{-1} + \bar{EI}^{-1}]^{-1}}{[\bar{E}^{-1} + \bar{I}^{-1} + \bar{EI}^{-1}]^{-1} [\bar{E}^{-1} + \bar{D}^{-1} + \bar{ED}^{-1}]^{-1}} \Delta\varepsilon_1 \Delta V_1 \\
 &+ \frac{[\bar{E}^{-1} + \bar{D}^{-1} + \bar{ED}^{-1}]^{-1}}{[\bar{E}^{-1} + \bar{I}^{-1} + \bar{EI}^{-1}]^{-1} [\bar{E}^{-1} + \bar{D}^{-1} + \bar{ED}^{-1}]^{-1}} \Delta\varepsilon_1 \Delta V_2
 \end{aligned}
 \tag{8}$$

The reaction volumes for this system can be determined conveniently by carrying out a titration in which  $\Delta A^*$  is measured following each addition of inhibitor I to an equilibrium mixture of E and D. A simulated titration curve is given in Fig. 1.

Fitting experimental absorbance changes to eq. 8 with a two-parameter least-squares program will yield the products  $\Delta\varepsilon_1 \Delta V_1$  and  $\Delta\varepsilon_1 \Delta V_2$ . Since  $\Delta\varepsilon_1$  can be determined independently, values for both volume changes can be obtained from a single titration.

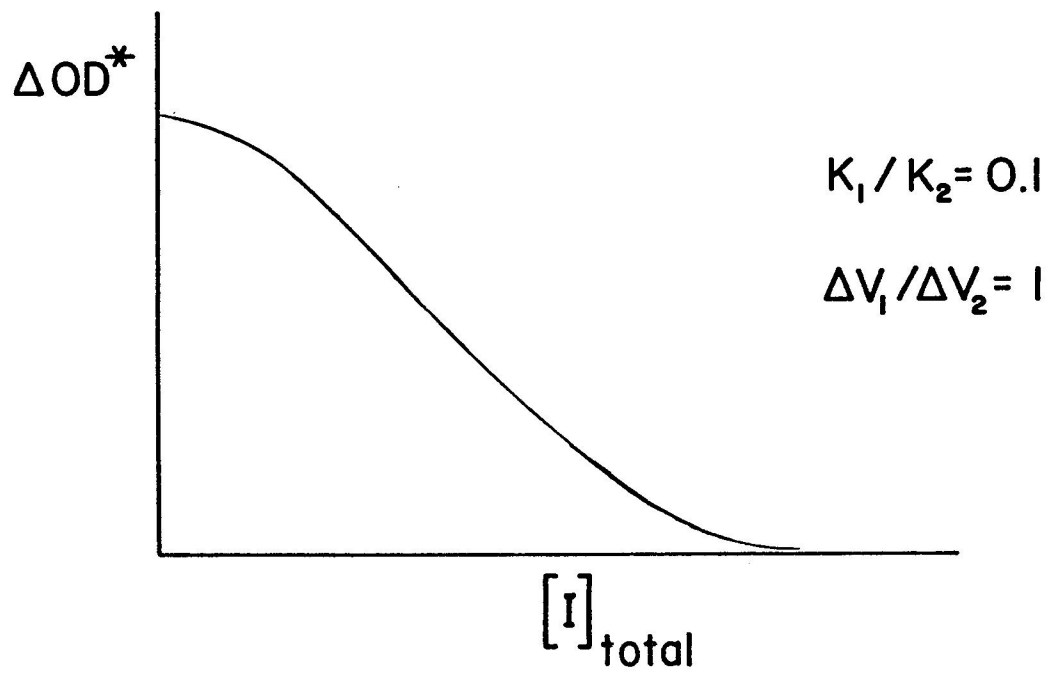


FIGURE 1

For more complex coupled equilibria, generation of the inverse matrix by either algebraic or numerical methods will still allow use of linear regression analysis to find all the reaction volumes. Even with several coupled equilibria, it is, only necessary to perform one relatively simple titration experiment at high pressure to calculate all the volume changes.

### Footnotes

- a) The "small perturbation" condition is defined by the relation

$$\delta K_i / K_i = -(\Delta V_i / RT) \delta P \leq 0.1$$

where  $K_i$  are elementary equilibrium constants.

- b)  $\Delta A^*$  is the absorbance change normalized with respect to temperature and the pressure change.

The general form of equation 1 is

independent of the forcing function

(temperature, pressure, etc.) and the

detection system.

$$\Delta A^* = (RT/\delta P) \Delta A$$

### References

1. D. Thusius, G. Foucault and F. Guillain in Dynamic Aspects of Conformation Changes in Biological Macromolecules, C. Sadron, ed., Reidel, Boston, 1973, pp. 271-284.
2. D. Thusius, in Chemical and Biological Applications of Relaxation Spectrometry, E. Wyn - Jones, ed., Reidel, Boston, 1975, pp. 113-119.
3. D. Thusius, *Biophys. Chem.*, in press.