Thermochimica Acta, 184 (1991) 117–124
Elsevier Science Publishers B.V., Amsterdam

Flow microcalorimetric study of the 1 : 1 stoichiometric reaction

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(Received 26 October 1990)

Abstract

A new mathematical treatment is presented for determination of the enthalpy change and equilibrium constant for the 1 : 1 stoichiometric reaction using flow microcalorimetric data. A reaction for which the thermodynamic parameters are known has been carried out in order to demonstrate the applicability of the method. The advantage of the proposed method is that the thermodynamic values are calculated directly by solving a biquadratic equation involving only measured experimental quantities, with no initial estimates being required. A dilution cell has been incorporated in the experimental set-up so that the concentration of one of the reactants could be varied continuously. It is also shown that the volume of the dilution cell may be directly determined with a flow microcalorimetric experiment.

INTRODUCTION

Ligand binding reactions of 1 : 1 stoichiometry are of general interest in many chemical and, particularly, biochemical applications [1]. Calorimetric methods [2], including flow [3,4] and titration [5] calorimetry, have been applied to the study of these reactions. However, all these methods require an initial estimation of the desired quantities followed by iterative calculation. It would be useful to be able to directly calculate the thermodynamic quantities, so we have developed equations to calculate the enthalpy change ΔH and equilibrium constant K from flow calorimetric data. This paper presents a new mathematical approach to the treatment of flow calorimetric data for 1 : 1 reactions and the incorporation of a dilution cell for continuous variation of concentration for one of the reactants.

THEORY

For any 1 : 1 stoichiometric reaction

$$A + B \rightleftharpoons C \qquad (1)$$

0040-6031/91/$03.50

the enthalpy of the reaction may be obtained from flow microcalorimetric data as follows:

$$\Delta H = \frac{P}{cR} \tag{2}$$

where R is the sum of the flow rates of the two reactants, c is the equilibrium concentration of the reaction product C, and P is the rate of heat production (mW) due to the formation of C. Experimentally, P is measured from

$$P = DE \tag{3}$$

where D is the chart recorder displacement (mm) and E is the electrical calibration constant (mW mm^{-1}).

The equilibrium constant for the binding reaction may be written as

$$K = \frac{c}{(a-c)(b-c)} \tag{4}$$

where a and b are the initial concentrations of A and B. Equation (4) can be rearranged as follows:

$$\frac{1}{K} = \frac{ab}{c} - (a+b) + c \tag{5}$$

Combining eqn. (5) with eqn. (2)

$$\frac{1}{K} = \frac{abR}{P}\Delta H - (a+b) + \frac{P}{R}\frac{1}{\Delta H} \tag{6}$$

Putting $\alpha = abR/P$, $\beta = a + b$, $\gamma = P/R$, eqn. (6) becomes

$$\frac{1}{K} = \alpha\,\Delta H - \beta + \frac{\gamma}{\Delta H} \tag{7}$$

and eqn. (7) may be written

$$\beta = \alpha\,\Delta H - \frac{1}{K} + \frac{\gamma}{\Delta H} \tag{8}$$

Equation (8) contains three variables, α, β, γ, and two constants, ΔH, K. We apply the least-squares method, and obtain the following two equations:

$$\frac{1}{K} = \frac{1}{N}\left(\Delta H \Sigma\alpha_i - \Sigma\beta_i + \frac{\gamma_i}{\Delta H}\right) \tag{9}$$

$$\frac{1}{K} = \frac{\Delta H^3 \Sigma\alpha_i - \Delta H^2 \Sigma\alpha_i\beta_i + \Sigma\beta_i\gamma_i - (\Sigma\gamma_i/\Delta H)}{\Delta H^2 \Sigma\alpha_i - \Sigma\gamma_i} \tag{10}$$

where N is the number of experimental points in a set of experiments. Combining eqn. (9) and eqn. (10), establishes a biquadratic equation in ΔH

$$\Delta H^4\left(N\Sigma\alpha_i^2 - \Sigma^2\alpha_i\right) - \Delta H^3\left(N\Sigma\alpha_i\beta_i - \Sigma\alpha_i\Sigma\beta_i\right) + \Delta H\left(N\Sigma\beta_i\gamma_i - \Sigma\beta_i\Sigma\gamma_i\right) - \left(N\Sigma\gamma_i^2 - \Sigma^2\gamma_i\right) = 0 \tag{11}$$

The roots may be directly calculated [6] and the most reasonable one selected. K can then be calculated from eqn. (9) or eqn. (10).

After both ΔH and K are obtained, a fitting curve may be drawn and compared with the experimental data. From eqn. (4), c may be written as

$$c = \frac{\left(a + b + \frac{1}{K}\right) \pm \left[\left(a + b + \frac{1}{K}\right)^2 - 4ab\right]^{1/2}}{2} \tag{12}$$

Because $c < (a + b)/2$, the root with the negative sign before the square root is reasonable. Substituting for c from eqn. (2)

$$P = \Delta HR \frac{\left(a + b + \frac{1}{K}\right) - \left[\left(a + b + \frac{1}{K}\right)^2 - 4ab\right]^{1/2}}{2} \tag{13}$$

In a titration experiment one of the reactant concentrations, b for example, is kept constant and the other, a, is varied. The experimental data may be graphically expressed as P vs. a, and the sum of squares of residuals, SSR, can be calculated to compare the goodness of fit.

If K is very large eqn. (12) becomes

$$c = \frac{(a + b) - |a - b|}{2} \tag{14}$$

When $a < b$, $c = a$

$$\Delta H = \frac{P}{aR} = \frac{P_m}{R} \tag{15}$$

where $P_m = P/a$, the slope of the initial part of the graph. For $a > b$, $c = b$

$$\Delta H = \frac{P}{bR} = \frac{P_m}{R} \tag{16}$$

where $P_m = P/b$, the plateau value of the last part of the graph divided by fixed concentration b.

DILUTION CELL METHOD

The reactant concentration may be varied incrementally (serial method) or continuously (dilution cell method). The dilution cell method, its application and advantages have previously been described [4,7]. In the dilution cell, the concentration of reactant A increases in an exponential manner

$$a = a_0\{1 - \exp[-r(t + t_0)]\} \tag{17}$$

where a_0 is the concentration of reactant A entering the dilution cell; t_0 is the time at which solution A reaches the dilution cell containing the solvent (when the concentration of A in the dilution cell is zero); $r = R_a/V$, where R_a is the flow rate of solution A, and V is the volume of the dilution cell.

V may be determined by analytical techniques such as spectrophotometry and t_0 may be accounted for theoretically [7]. However, an easy and effective method is to carry out a known reaction on the flow microcalorimeter itself. If the reaction has quite a large K value, from eqn. (15), eqn. (17) may become

$$P = P_0\{1 - \exp[-r(t + t_0)]\} \tag{18}$$

P_0 is the rate of heat production corresponding to a_0. In this case, t_0 is the time when the front of solution A reaches the reaction cell of the microcalorimeter. From the straight line plot of $\ln(P_0 - P)$ vs. t, V and t_0 can be obtained.

EXPERIMENTAL METHODS AND RESULTS

Figure 1 illustrates the instrumental set-up. The LKB 2107 Flow Microcalorimeter C has previously been described [8]. The pump D, an LKB 2132 Microperpex, has dual channels and the flow rates were shown to remain constant daily within 0.0003 ml min^{-1} after continuous running for more than 24 h. The dilution cell E, which was used in the dilution cell method, consisted of a beaker, a rubber stopper with two metal tubes and a magnetic stirrer.

The reaction of an *o*-bromoaniline (BA) solution with hydrochloric acid has been extensively studied by titration calorimetry [9]. This reaction was performed keeping the BA concentration constant (ca. 10 mM before mixing) and serially varying the HCl concentration (from 0 to 100 mM before mixing). The baseline referred to the mixing of the BA solution with water. The heat of dilution of each HCl solution was measured by performing a separate blank experiment. The experimental quantities, including the HCl concentration a, the rate of heat production P corresponding to each a, the number of HCl solutions N, the BA concentration and the flow rates of both solutions, were employed in eqn. (11). Each root was used to calculate K from eqn. (9) and then to fit eqn. (13) but only one root was reasonable. By computer program the entire calculation took less than one minute. One set of experimental data and its fitting curve is shown in Fig. 2

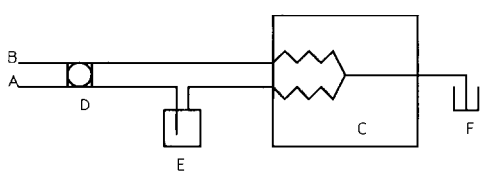

Fig. 1. Schematic diagram of instrument set-up: A, varying concentration solution inlet; B, constant concentration solution inlet; C, calorimeter; D, pump; E, mixing cell; F, waste.

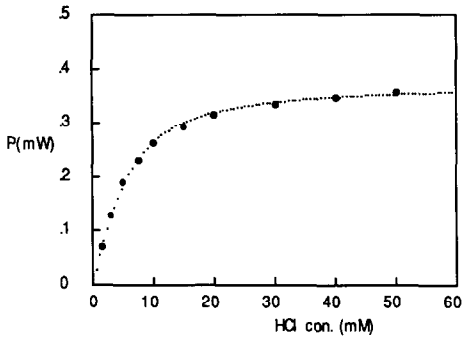

Fig. 2. Flow microcalorimetric data and their fitting curve for the reaction of *o*-bromoaniline with HCl.

whilst the results of four duplicate experiments and the standard values are listed in Table 1.

These results demonstrate both the applicability of the new mathematical method and the accuracy of the flow microcalorimeter.

On attempting the use of a dilution cell in flow microcalorimetry, it was found that the consideration of the dilution cell volume V and the starting time t_0 was critical. t_0 could not be easily located on the chart paper because the recorded curve near t_0 was not perfect. V was best measured by flow microcalorimetry in a separate experiment. The reaction of THAM (tris(hydroxymethyl)aminomethane) and HCl was carried out to measure V and t_0.

The THAM solution was freshly prepared at 4.392 mM and the HCl concentration was 15.00 mM. The THAM solution was pumped through channel B at the rate of 0.1189 ml min^{-1}, its dilution effect due to mixing being accounted for in the baseline. The HCl solution was diluted in the dilution cell and passed through channel A at the rate of 0.1216 ml min^{-1}. The THAM reacting concentration was 2.171 mM and the HCl initial concentration after mixing was 7.584 mM. The recorded experimental curve is illustrated in Fig. 3. In this case the HCl dilution effect due to mixing was shown to be negligible in a separate blank experiment.

P_0 does not show directly on the curve. The plateau $P' = 0.4092$ mW correponds to the HCl concentration 2.171 mM, which is equal to the

TABLE 1

Flow microcalorimetric results and standard data for the reaction of *o*-bromoaniline with hydrochloric acid

	Results	Literature [9]
$-\Delta H$ (kcal mol^{-1})	4.39 ± 0.09 [a]	4.39 ± 0.07
log K	2.56 ± 0.03	2.76

[a] The errors are expressed as standard deviations.

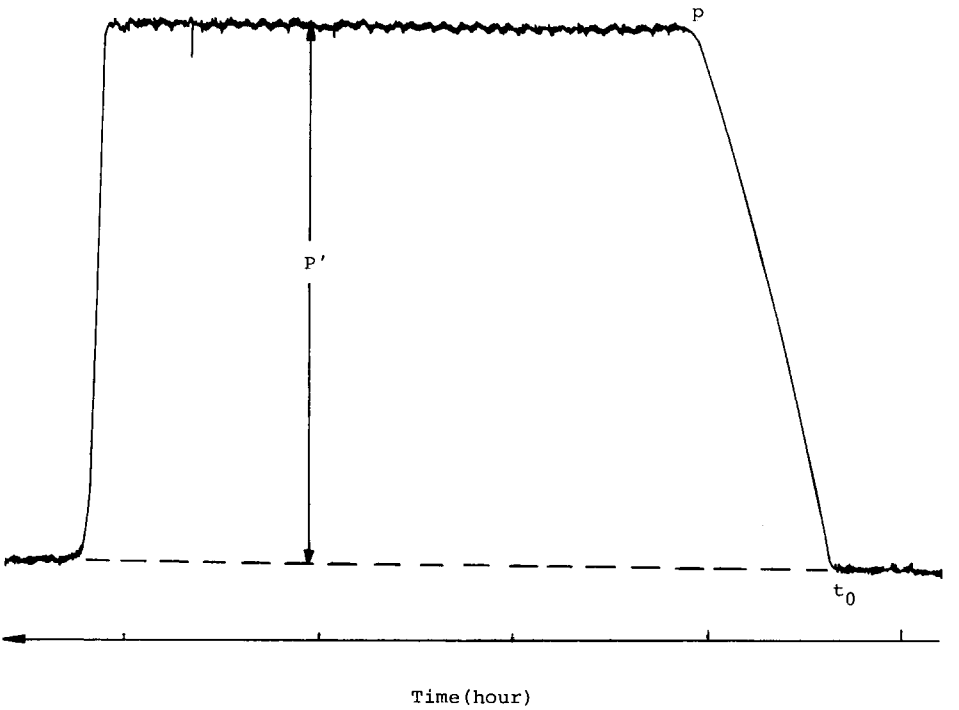

Fig. 3. Flow microcalorimetric curve for the reaction of THAM with HCl.

constant concentration of THAM. From this relationship P_0 may be calculated:

$$P_0 = (7.584/2.171)0.4092 = 1.429 \text{ mW}$$

The calorimetric response is measured in terms of recorder chart paper divisions. The data preceding the inflection point p are used to generate the graph of $\ln(P_0 - P)$ versus t, Fig. 4, and from the straight line V and t_0 are obtained.

The volume of the dilution cell was determined in four experiments and shown to be 12.15 ± 0.02 ml.

The concentrations corresponding to P values are calculated according to eqn. (17); ΔH and K may then be obtained using eqn. (11) and eqn. (9). ΔH is determined with good precision but K is strongly dependent on the value of t_0, even if t_0 varies by just 0.01 minute. The experiments were repeated four times, and the best precision is obtained when t_0 is chosen to yield the minimum value of SSR in a 0.03 minute range of the t_0 value obtained from the staight line. The results are: $\Delta H = -11.19 \pm 0.03$ (kcal mol^{-1}), $\log K = 6.45 \pm 0.04$. The value of ΔH compares favorably with literature data ($\Delta H = -11.33$ kcal mol^{-1}) [10]. This also shows the accuracy of our flow microcalorimeter. The K value is considerably smaller (literature value $\log K = 8.07$). The reason lies in the inherent limitation of the calori-

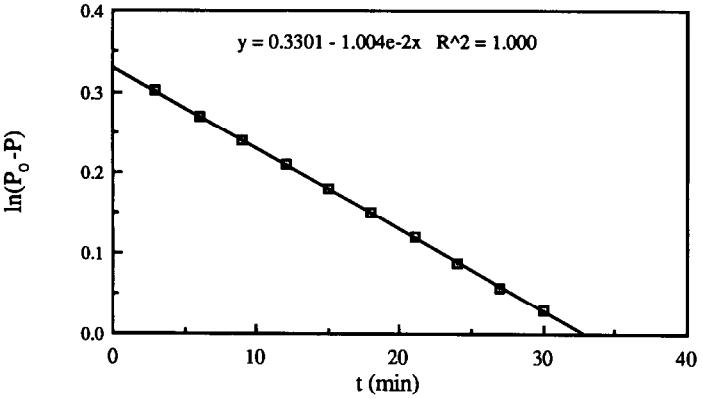

Fig. 4. Straight line for obtaining V and t_0.

metric method for determination of large values of K [2]. The problem is readily visualized with eqn. (9). The best precision obtainable for β_i is 0.01 mM due to the variation of flow rates. For $N = 30$, for example, the largest log K is about 6.

If the only aim is the determination of V, the experiment may be carried out more easily by keeping the HCl concentration constant and varying the THAM concentration (the HCl concentration is higher than that of THAM). In this case, P_0 can be directly shown on the experimental curve by pumping the THAM solution for a period of time without passing it through the dilution cell, and ΔH can be obtained from eqn. (15).

Generally, when flow microcalorimetry is employed for practical use, its accuracy should first be checked by a standard reaction. If the dilution cell method is incorporated, V and t_0 have to be measured. The reaction of THAM with HCl is useful for this purpose.

An example of the practical application of the mathematical treatment and the dilution cell method presented here is given in Table 2 for the complexation of sodium cholate (C) by β-cyclodextrin (β-CD) in Tris (trishydroxylmethylaminomethane) buffer solution. The results are identical for the serial method and dilution cell method within the limits of experimental error.

TABLE 2

Results of the reaction of β-cyclodextrin (β-CD) with sodium cholate (C) in pH 7.4 Tris buffer

$-\Delta H$ (kJ mol^{-1})	K (10^3 M)	Buffer con. (mM)	Method
25.8 ± 0.2	3.1 ± 0.2	25	cell
25.6 ± 0.3	3.6 ± 0.3	50	cell or serial
25.4 ± 0.3	3.1 ± 0.3	75	serial

ACKNOWLEDGEMENTS

The authors would like to thank Mr. Hong Qi for helpful discussions concerning the design of the dilution cell. The authors are also grateful to Marion-Merrel Dow for partial support of this work.

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