

# Calorimetric vs. van't Hoff binding enthalpies from isothermal titration calorimetry: Ba<sup>2+</sup>-crown ether complexation

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## Abstract

The 1:1 complexation reaction between Ba<sup>2+</sup> and 18-crown-6 ether is re-examined using isothermal titration calorimetry (ITC), with the goal of clarifying previously reported discrepancies between reaction enthalpies estimated directly (calorimetric) and indirectly, from the temperature dependence of the reaction equilibrium constant  $K$  (van't Hoff). The ITC thermograms are analyzed using three different non-linear fit models based on different assumptions about the data error: constant, proportional to the heat and proportional but correlated. The statistics of the fitting indicate a preference for the proportional error model, in agreement with expectations for the conditions of the experiment, where uncertainties in the delivered titrant volume should dominate. With attention to proper procedures for propagating statistical error in the van't Hoff analysis, the differences between  $\Delta H_{\text{cal}}$  and  $\Delta H_{\text{vH}}$  are deemed statistically significant. In addition, statistically significant differences are observed for the  $\Delta H_{\text{cal}}$  estimates obtained for two different sources of Ba<sup>2+</sup>, BaCl<sub>2</sub> and Ba(NO<sub>3</sub>)<sub>2</sub>. The effects are tentatively attributed to deficiencies in the standard procedure in ITC of subtracting a blank obtained for pure titrant from the thermogram obtained for the sample. © 2003 Elsevier B.V. All rights reserved.

**Keywords:** Isothermal titration calorimetry (ITC); Data analysis; Non-linear least squares; Ba<sup>2+</sup>/crown ether complexation; van't Hoff reaction enthalpy; Calorimetric vs. van't Hoff  $\Delta H$

## 1. Introduction

In isothermal titration calorimetry (ITC), the heat  $q$  is measured for incremental injection of one reactant (the titrant) into a reaction vessel containing the other reactant (the titrate, often a macromolecular substance in biochemical applications). The data thus form a titration curve of heat vs. extent of reaction, the

analysis of which yields  $\Delta H_{\text{cal}}$  (the calorimetric reaction enthalpy) and the equilibrium constant  $K$  [1–3]. Repetition of the experiment over a range of temperatures yields  $\Delta H_{\text{cal}}(T)$  and  $K(T)$ . The latter can be further analyzed using the van't Hoff relation,

$$\left(\frac{\partial \ln K^\circ}{\partial T}\right)_P = \frac{\Delta H^\circ}{RT^2}, \quad (1)$$

to yield a second estimate of  $\Delta H$  for the reaction (van't Hoff,  $\Delta H_{\text{vH}}^\circ$ ).

In recent years there has been controversy over inconsistencies between the calorimetric and van't

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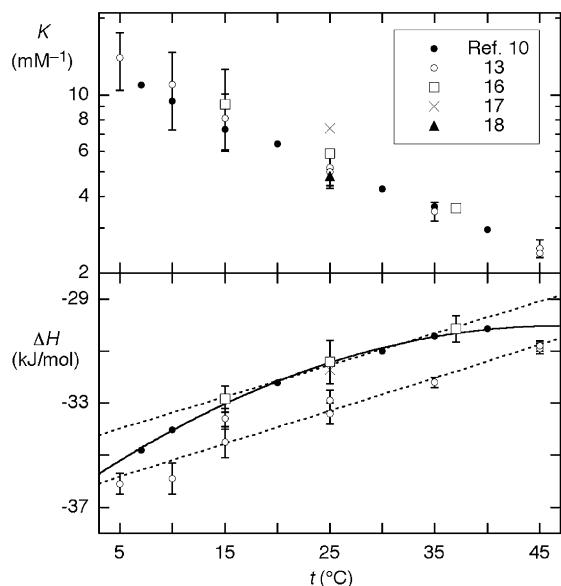
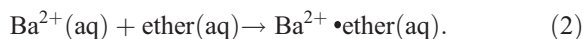


Fig. 1. Literature results for  $K$  and  $\Delta H_{\text{cal}}$  for the complexation of  $\text{Ba}^{2+}$  by 18-crown-6 ether in water. Note log scale for  $K$ . Error bars represent reported standard errors, which are comparable to the size of the displayed points for  $K$  from Ref. [10]. Results from Refs. [17,18] were reported only at 25 °C (no  $\Delta H$  for the latter). In the lower frame, the solid curve is a quadratic fit to the data from Ref. [10] while the dashed lines represent linear fits to the data from Ref. [13] (lower) and [16]. (This is fig. 12 from Ref. [15], with modifications.)

Hoff (vH) estimates of  $\Delta H$  obtained using ITC [4–14]. There can be little doubt of the validity of Eq. (1), because it follows directly from the purely mathematical Gibbs–Helmholtz equation. Both Chaires [12] and Horn et al. [13] have noted that the precision of the  $\Delta H_{\text{vH}}$  estimates is considerably lower than that for  $\Delta H_{\text{cal}}$  and have attributed the discrepancies to problems associated with this reduced precision. However, a closer examination of the data for several test cases, with proper attention to error propagation in assessing the uncertainties in  $\Delta H_{\text{vH}}$ , shows that most of the literature discrepancies between  $\Delta H_{\text{cal}}$  and  $\Delta H_{\text{vH}}$  remain uncomfortably large [15].

A key test reaction in such work, and one that has sometimes been used to calibrate ITC instruments, is the complexation of  $\text{Ba}^{2+}$  with 18-crown-6 ether [10,13,16–18],



As Fig. 1 shows, the disparities among the various experimental studies of this reaction often

exceed the apparent precisions from the experiments, for both  $K$  and  $\Delta H_{\text{cal}}$ . Also, the most precise data, from Ref. [10], show clear curvature in the dependence of  $\Delta H_{\text{cal}}$  on  $T$ , while the other determinations do not. A vH analysis of the data from Ref. [10] also substantiates the claim of disagreement by the authors, as is shown in Fig. 2. While Horn et al. concluded that their  $\Delta H_{\text{cal}}$  and  $\Delta H_{\text{vH}}$  estimates were consistent [13], a reexamination of their vH analysis indicates that at best, agreement is salvaged through unusually large uncertainties in  $K$  and in the resulting  $\Delta H_{\text{vH}}$  estimates [15].

In the present work we have reexamined this  $\text{Ba}^{2+}$  complexation reaction with the aim of increasing the precision of measurement so as to more clearly define the disagreement between calorimetric and van't Hoff estimates of  $\Delta H$ . To aid in understanding any inconsistencies, we have used two sources of  $\text{Ba}^{2+}$ —the customary  $\text{BaCl}_2$ , and for the first time,  $\text{Ba}(\text{NO}_3)_2$ . These two have rather different dissolution heat properties, which could be relevant if artifacts associated with this property are involved in the discrepancies. In assessing the uncertainties in the  $\Delta H_{\text{vH}}$  values, we properly account for the correlation in the fit parameters [15,19]. We also analyze our data using three different fit models: the customary constant error model, a proportional error model and a correlated error model [20]. The result-

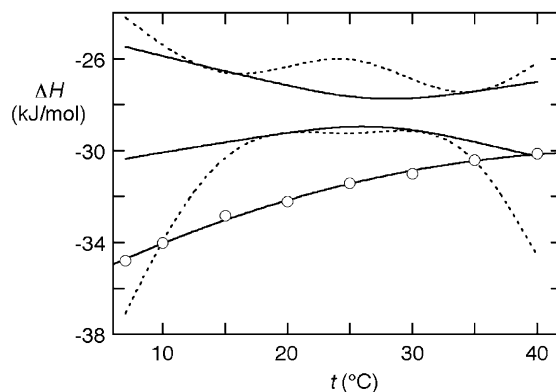


Fig. 2.  $\Delta H_{\text{cal}}$  estimates from Liu and Sturtevant [10] for  $\text{Ba}^{2+}$ /ether complexation (points and their quadratic least-squares representation), and 1- $\sigma$  error bands on van't Hoff estimates using quadratic (dashed curves) and linear (solid) representations for  $\Delta H$  as a function of  $T$ . (details of error band calculations given below; this is fig. 13 from Ref. [15], modified).

ing model error in  $\Delta H_{\text{vH}}$  is comparable to the statistical error. Both are too small to account for the differences between the calorimetric and vH estimates. To explain the discord, we suggest several effects that have not been considered previously.

## 2. Methods

### 2.1. Thermodynamic model

Eq. (1) is often written in the alternative form,

$$\Delta H^\circ = -R \left( \frac{\partial \ln K^\circ}{\partial (1/T)} \right)_p \quad (3)$$

from which a linear dependence of  $\ln K^\circ$  on  $T^{-1}$  implies a constant  $\Delta H^\circ$ . It is important to note that the ‘°’ on  $K^\circ$  and  $\Delta H^\circ$  means that these quantities are referenced to their standard states [21]. As was noted earlier, Eq. (3) is exact, essentially by definition. It is customary to use concentration  $K$ s in ITC, so  $K^\circ$  is the dimensionless version of  $K_c$  (units l/mol here), with activities in place of concentrations; and  $\Delta H^\circ$  refers to the conversion of reactants in their standard states to products in theirs. These are the fictitious infinite dilution states at  $c=1.0$  mol/l. In fact, for concentration reference states, Eq. (1) requires a minor clarification; for reaction (2) it reads

$$\left( \frac{\partial \ln K^\circ}{\partial T} \right)_p = \frac{\Delta H^\circ}{RT^2} + \alpha(T), \quad (1a)$$

where  $\alpha(T)$  is the thermal expansivity of the solvent (see problem 11.35 in Ref. [21]). In this paper we omit the ‘°’ from all experiment-based quantities that are intended to approximate the thermodynamic ideal, to acknowledge that the two may not be identical. The task, then, is to explain the difference.

Solution reactions often display significant  $T$ -dependence in  $\Delta H$  over even small ranges of temperature. In all of the work below,  $\Delta H^\circ$  is assumed to be expressible as quadratic in  $T$  over the range encompassed by the data,

$$\Delta H^\circ = a + b(T-T_0) + c(T-T_0)^2. \quad (4)$$

From this expression, at  $T=T_0$ ,  $\Delta H^\circ=a$ ,  $\Delta C_P^\circ=b$  and  $d\Delta C_P^\circ/dT=2c$ . Integration of Eq. (1) then yields

$$R \ln(K^\circ/K_0^\circ) = A \left( \frac{1}{T_0} - \frac{1}{T} \right) + B \ln \left( \frac{T}{T_0} \right) + c(T-T_0), \quad (5)$$

where

$$A = a - bT_0 + cT_0^2, \quad \text{and} \quad B = b - 2cT_0. \quad (6)$$

A set of data for  $K^\circ(T)$  can be fitted to Eq. (5) or its exponential form using non-linear LS, yielding  $K_0^\circ$ ,  $a$  and (if assumed significant)  $b$  and  $c$ . Since the  $K^\circ$  values are ordinarily determined with varying precision, such a fit is properly a weighted fit, with the actual weights depending on whether the logarithmic or exponential form is fitted [15,22].

To include the effect of the correction term in Eq. (1a), it is necessary to express the thermal expansivity of the solvent (water) as a function of  $T$ . If the density is taken as

$$\rho(T) = \rho_r \exp[-h(T, T_r)], \quad (7)$$

where  $\rho_r$  is the density at some reference temperature  $T_r$ , and where the function  $h(T, T_r)$  is designed to go to zero at  $T_r$ , then  $\alpha=dh/dT$ . For example a polynomial in  $x=(T-T_r)$ , missing the constant term, works here; and we find that a fourth-order polynomial for  $h$  adequately represent the density of water over the range 5–50 °C. With this treatment, the correction term in Eq. (1a) leads to an additional term  $Rh(T, T_r)$  on the right-hand side of Eq. (5), in which all coefficients are known.

### 2.2. Non-linear least-squares fit models

The least-squares (LS) analysis of ITC data has been considered in detail recently [15,20] and will be reviewed only briefly here. There are three limiting fit models. The commonly used constant error model (or unweighted LS) is appropriate when the statistical error in the data is dominated by a constant uncertainty in the measurement of the heat  $q$ . If the data error is dominated by uncertainty in the delivered

volume of titrant, the error in  $q$  is proportional to  $q$ , requiring weighting of the  $i$ th point proportional to  $q_i^{-2}$ . That, however, assumes that the volume error is random in  $v$ . If it is the accumulated titrant volume after  $i$  injections that is subject to random error, the incremental volume is the difference of two such quantities and hence possesses correlated error, requiring analysis by correlated LS. The constant error model should be appropriate for low- $q$  experiments. The  $\text{Ba}^{2+}$ /crown ether complexation is not in this category, so one expects either proportional error or correlated error to dominate here.

All fit models include, in addition to  $K$  and  $\Delta H$ , the ‘site number’  $n$  [1]. For simple 1:1 binding like that involved here,  $n$  is simply a concentration correction factor [15,20]; it is applied to the titrate concentration, with the assumption that the prepared titrant concentration is known exactly. In recent work we have noted that typical ITC procedures can yield data afflicted with a systematic shortfall of titrant, due to backlash in the motorized screw drive on the syringe plunger [23]. Since most of our data were collected following the suspect procedure, they were fitted as in Ref. [23], with an additional parameter representing the titrant volume error in the first injection.

For the weighted fit models, we know the data errors in only a relative sense, so we use the customary a posteriori variance–covariance matrix to estimate the parameter standard errors [15,20],

$$\mathbf{V} = \frac{S}{N - p} \mathbf{A}^{-1}. \quad (8)$$

Here  $S$  is the sum of weighted squared residuals,  $N$  is the number of fitted  $q_i$  values,  $p$  the number of adjustable parameters (4 when the volume correction is included), and  $\mathbf{A}$  is the Hessian matrix. The parameter variances are the respective diagonal elements of  $\mathbf{V}$ . These a posteriori error estimates have drawbacks when used to weight the results in a subsequent analysis [24], e.g. the vH analysis of a set of  $K$  values over a range of  $T$ . However, lacking a reliable assessment of the inherent data error, we can either just neglect weights in the vH analysis or weight using these estimates; we have checked results both ways.

### 2.3. Statistical error propagation

The textbook expression,

$$\sigma_f^2 = \sum \left( \frac{\partial f}{\partial \beta_j} \right)^2 \sigma_{\beta_j}^2, \quad (9)$$

is normally used to compute the propagated error in some function  $f$  of the independent variables  $\beta$ , where the sum runs over all uncertain variables  $\beta_j$ . However, Eq. (9) assumes that these variables are uncorrelated. This assumption seldom holds for a set of parameters  $\beta$  returned by an LS fit, and one must use the more general expression,

$$\sigma_f^2 = \mathbf{g}^T \mathbf{V} \mathbf{g}, \quad (10)$$

in which the  $j$ th element of the vector  $\mathbf{g}$  is  $\partial f / \partial \beta_j$ . This expression is rigorously correct for functions  $f$  that are linear in variables  $\beta_j$  that are themselves normal variates [19]. For non-linear functions of normal and non-normal variates, its validity is limited by the same 10% rule of thumb that applies to parameters estimated by non-linear LS [22]: If the  $\mathbf{V}$ -based standard error is less than 1/10 the magnitude of the parameter, then the Gaussian interpretation of this error should suffice within 10% to define the confidence limits. In fact, often the computation of  $\sigma_f$  can be facilitated by simply redefining the fit function so that  $f$  is one of the adjustable parameters of the fit [15,19].

In the present case, the quantity of primary interest is  $\Delta H_{\text{vH}}^\circ$ , as defined by Eq. (4) and obtained by non-linear fitting of  $K^\circ(T)$  to Eq. (5). The parameters  $a$ ,  $b$  and  $c$ , will usually be at least partially correlated, meaning Eq. (10) is needed for proper computation of the error. However, since these three parameters have special significance at  $T=T_0$ , the propagated errors in  $\Delta H^\circ$  and  $\Delta C_p^\circ$  can be obtained over the desired range of  $T$  by simply repeating the fit for a selection of  $T_0$  values spanning this range. This procedure is easily implemented with data presentation and analysis programs like KaleidaGraph (Synergy Software) [25], whereas Eq. (10) is not. The error bands for  $\Delta H_{\text{vH}}^\circ$  in Fig. 2 were generated in this manner.

## 2.4. Experiments

The reaction was studied at temperatures between 6.4 and 47.0 °C, using a MicroCal VP-ITC instrument having a cell volume of 1.41 ml. The 18-crown-6 (Aldrich, 99%) was taken as titrate in most experiments, while BaCl<sub>2</sub>·2H<sub>2</sub>O (MCB, reagent grade) or Ba(NO<sub>3</sub>)<sub>2</sub> (Fisher, certified) was used as titrant. The cell and syringe were always rinsed at least twice with fresh solution prior to filling. In the runs at temperatures away from room *T*, the reagents were also pre-thermostatted to approximately the cell temperature. Solution concentrations decrease by ~1% between 6 and 47 °C, due to the thermal expansion of water; results were corrected for this effect, as described below.

Theoretically, fewer injections should give better precision (except for correlated data) [20,26]. Accordingly most runs used only nine or 10 injections (including an initial discard) and spanned a stoichiometry range (titrant/titrate) of 2–3. To ensure near-complete return to baseline between injections, a relatively long injection interval (6 min) was used. In standard fashion, a blank was obtained by adding titrant to water using the same program sequence. The fitted data were then obtained by subtracting this blank from the raw data.

The use of a small number of injections means more heat per injection. This results in complications with a highly sensitive instrument like the VP-ITC and the very exothermic Ba<sup>2+</sup>/ether complexation reaction. The instrument was operated at higher bias levels than normal, to avoid negative compensation power (which leads to erroneous *q<sub>i</sub>* values). It was also necessary to work at low ether concentrations ([M]<sub>0</sub> ≈ 1 mM), such that *K* [M]<sub>0</sub> was typically 2–13, which is lower than generally considered optimal [1,27]. Still, we obtained estimates of *K* and Δ*H* with nominal standard errors that rarely exceeded 1% of the parameter magnitudes.

## 3. Results and discussion

### 3.1. Evidence for van't Hoff/calorimetric inconsistency

The data were analyzed using the three limiting fit models mentioned earlier: constant error, proportional

error and correlated error. Since titrant volume error is expected to dominate in the present experiments, and since subsequent fitting (discussed below) shows a preference for the proportional error model, we present results obtained with that model in Table 1. These have been corrected for the temperature dependence

Table 1  
ITC experiments on Ba<sup>2+</sup> complexation with 18-crown-6 ether<sup>a</sup>

<i>t</i> (°C)	[Ba <sup>2+</sup> ] (mM)	[ether] (mM)	<i>R<sub>m</sub></i> <sup>b</sup>	<i>K</i> <sup>c</sup> (10 <sup>3</sup> M <sup>-1</sup> )	–Δ <i>H</i> <sup>c</sup> (kJ/mol)	<i>n</i>
25.0	19.89	1.985	2.01	5.61 (2)	32.05 (6)	0.972
25.0	19.89	1.985	2.19	5.62 (2)	32.15 (6)	0.967
25.0	19.89	0.992	4.39	5.43 (3)	32.52 (21)	0.951
25.0	9.95	0.992	2.19	5.64 (3)	31.83 (9)	0.975
25.0	9.95	0.992	2.05	5.60 (1)	32.01 (4)	0.975
25.0	19.89	0.992	2.90	5.61 (2)	32.13 (11)	0.966
25.0	99.47	0.992	2.68	5.41 (1)	31.61 (6)	0.935
35.0	9.95	0.992	1.45	3.90 (5)	31.05 (17)	0.967
35.0	9.95	0.992	1.78	3.92 (2)	31.01 (10)	0.968
6.4	19.89	0.992	2.90	12.61 (6)	36.00 (14)	0.959
6.4	19.89	0.992	2.57	12.64 (7)	36.02 (14)	0.952
15.3	19.89	0.992	2.57	8.39 (5)	33.84 (14)	0.966
15.3	19.89	0.992	2.57	8.17 (9)	34.21 (27)	0.949
47.0	19.89	0.992	2.90	2.70 (1)	30.07 (15)	0.966
47.0	19.89	0.992	2.90	2.65 (2)	30.59 (22)	0.949
40.0	19.89	0.992	2.89	3.30 (2)	30.45 (13)	0.957
40.0	19.89	0.992	2.89	3.39 (5)	30.45 (41)	0.957
30.0	54.10	1.088	2.42	4.49 (3)	30.61 (22)	0.956
30.0	54.10	1.088	2.42	4.47 (4)	30.90 (19)	0.962
40.0	54.10	1.088	2.68	3.24 (2)	30.34 (19)	0.954
40.0	54.10	1.088	2.68	3.25 (2)	30.19 (16)	0.954
20.3	54.10	1.088	2.68	6.72 (3)	33.14 (15)	0.944
20.3	54.10	1.088	2.68	6.68 (4)	33.24 (20)	0.944
25.0	19.25	0.992	2.80	5.73 (2)	31.68 (10)	0.970
6.4	19.25	0.992	2.49	13.19 (6)	35.22 (11)	0.965
6.4	19.25	0.992	2.49	13.21 (6)	35.20 (11)	0.964
47.0	19.25	0.992	2.80	2.69 (2)	29.69 (17)	0.968
47.0	19.25	0.992	2.80	2.69 (2)	30.02 (20)	0.961
35.0	19.25	0.992	2.80	4.00 (2)	30.73 (14)	0.964
35.0	19.25	0.992	2.80	4.00 (2)	30.64 (14)	0.971
30.0	60.15	1.088	2.69	4.62 (3)	30.63 (16)	0.953
30.0	60.15	1.088	2.69	4.60 (2)	30.61 (13)	0.952
40.0	60.15	1.088	2.63	3.27 (3)	30.18 (22)	0.956
40.0	60.15	1.088	2.69	3.25 (2)	30.32 (18)	0.943
40.0	60.15	1.088	2.63	3.21 (2)	30.52 (14)	0.937
16.1	60.15	1.088	2.24	8.28 (5)	32.91 (12)	0.951
16.1	60.15	1.088	2.23	8.34 (3)	32.85 (08)	0.961

<sup>a</sup> First 23 runs using BaCl<sub>2</sub> as titrant, last 14 using Ba(NO<sub>3</sub>)<sub>2</sub>. Data analyzed using proportional error model and corrected for the temperature dependence of the density of water.

<sup>b</sup> Titration stoichiometry range, [Ba<sup>2+</sup>]/[ether].

<sup>c</sup> Figures in parentheses are standard errors, in terms of final displayed digits.



of the density of water, under the assumption that both titrant and titrate concentrations are subject to the same  $T$ -dependent concentration changes. Sample fits show that, when the titrant concentration is considered known,  $K$  and  $\Delta H$  are both inversely proportional to solvent density, so results can be processed using the nominal concentrations and corrected afterwards.

The correlated fit model gave only small changes in the estimated  $\Delta H$  and  $K$  values from those given in Table 1. Unweighted fitting gave larger differences, as much as 3% for  $K$  and 2% for  $\Delta H$ , with one significant pattern, illustrated in Fig. 3: The  $K$  values tended to be larger with this fit model for  $\text{BaCl}_2$  but smaller for  $\text{Ba}(\text{NO}_3)_2$ , while the opposite dependence was observed for the absolute  $\Delta H$  values (not shown). This behavior is possibly related to the calorimetric vs. vH discrepancies, as is discussed below. The three models gave more significant variations in the parameter standard error estimates, with the values in Table 1 being the most pessimistic for  $\sigma_{\Delta H}$  and intermediate for  $\sigma_K$ . Unweighted fitting gave the most optimistic estimates for  $\sigma_{\Delta H}$  and the most pessimistic for  $\sigma_K$ . These differences play a role in the statistics of subsequent fitting of  $\Delta H(T)$  and  $K(T)$  to functions of  $T$ , as discussed below.

The  $\Delta H$  values from Table 1 are illustrated in Fig. 4, together with the values from Liu and Sturtevant [10]. The chloride/nitrate differences are small, at

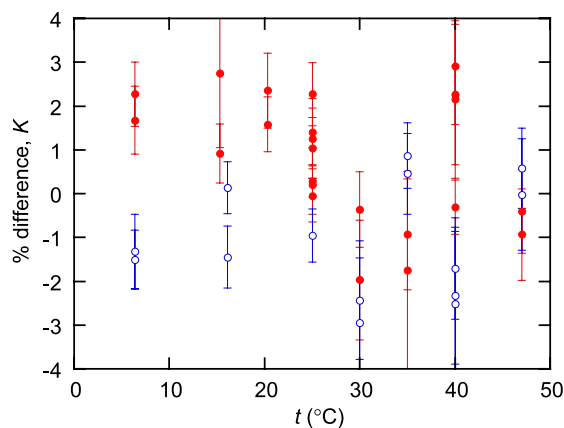


Fig. 3. Differences in  $K$  as estimated in fits with the constant-error model vs. the proportional error model, shown as percent change from the latter. Solid points— $\text{BaCl}_2$ , open— $\text{Ba}(\text{NO}_3)_2$ . Error bars represent  $1\sigma$  for the difference, also expressed as a percent of the result from the proportional error model.

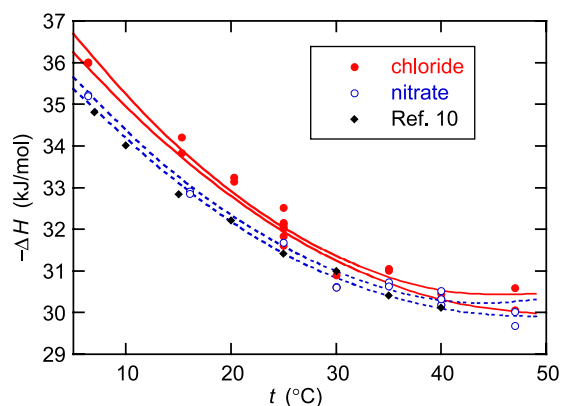


Fig. 4. Calorimetric  $\Delta H$  estimates from present work, compared with results of Liu and Sturtevant. Curves represent  $1\text{-}\sigma$  error bands from weighted LS fits to a quadratic function of  $t$  and yield  $\chi^2 = 96.5$  for  $\text{BaCl}_2$  (23 points) and 29.8 for  $\text{Ba}(\text{NO}_3)_2$  (14 points). The same treatment for results obtained using the correlated error model gave  $\chi^2 = 227$  and 75 for the two titrants, respectively, while the constant error model gave 303 and 100.

most only approximately 1 kJ/mol, however, they are statistically significant, as is clear from the illustrated statistical error bands. The  $\chi^2$  values are larger than they should be for these fits, by factors of 4.8 for  $\text{BaCl}_2$  and 2.7 for  $\text{Ba}(\text{NO}_3)_2$ . This is a reflection of the scatter in the values being considerably larger than the nominal standard errors for individual values in Table 1 and is an indication that problems in run-to-run repeatability exceed the apparent precisions from individual data sets. When these calculations were repeated for results obtained using the constant error model (unweighted fitting) and the correlated error model, the  $\chi^2$  values were larger still, by approximate factors of three and two, respectively. These comparisons indicate that the statistics from the latter models are even more unrealistic, and thus constitute our reason for judging the proportional error model to be best for these data. It should be noted these  $\chi^2$  values are  $S$  in Eq. (8), so use of the a posteriori definition of  $\mathbf{V}$  gives conservative estimates of the precisions (larger  $\sigma$ ) in cases like this, where  $\chi^2$  exceeds its expected value. Also, with this definition, the three fit models give more nearly comparable error bands on the fitted  $\Delta H(T)$  than might be suggested by their  $\chi^2$  values. Finally, the discord between the chloride and nitrate results in Fig. 4 cannot be removed by selecting one of the other fit models,

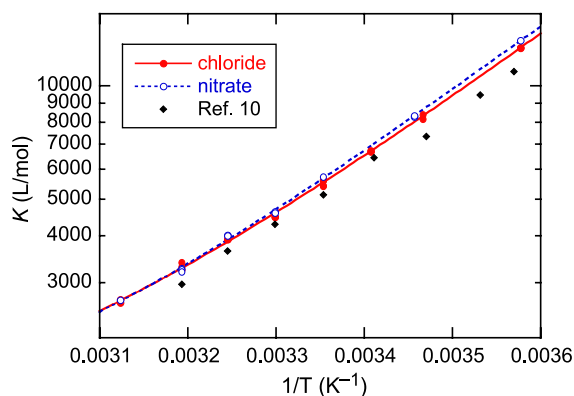


Fig. 5. Estimated  $K$  values and their LS representations from fits to Eq. (5), and values from Ref. [10]. The  $\chi^2$  values from these weighted fits were 213 for chloride and 92 for nitrate. For comparison, the correlated fit model gave 588 and 234, while the constant-error model gave 242 and 130.

because the model error is only approximately 1/5 the difference at low  $t$ , where the disparity is greatest in Fig. 4.

Fig. 5 illustrates the  $K$  values from Table 1 and their representation from weighted LS fits to Eq. (5). Again the  $\chi^2$  values are ‘too large,’ and again the proportional error model gives lowest values, though the distinction is not as pronounced here as in Fig. 4. The difference between the chloride and nitrate results exceeds two combined sigmas over much of the displayed range of  $T$  and thus appears to be statistically significant. However, in this case use of the

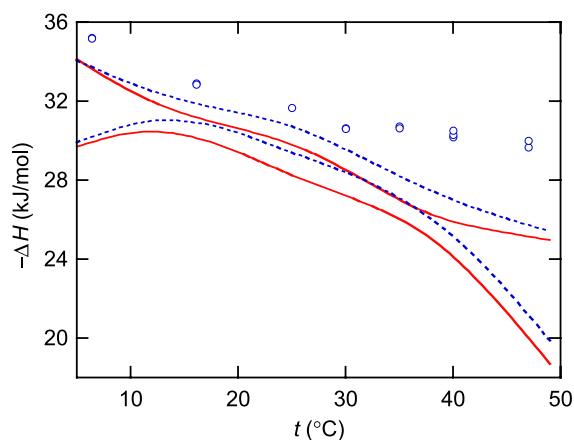


Fig. 6. Error bands delimiting 1- $\sigma$  region of  $\Delta H_{\text{vH}}$  for  $\text{BaCl}_2$  (solid) and  $\text{Ba}(\text{NO}_3)_2$  (dashed). Points are  $\Delta H_{\text{cal}}$  estimates for  $\text{Ba}(\text{NO}_3)_2$ .

constant-error model makes the difference vanish over much of the displayed curve, so we are reluctant to conclude that the difference is real. In any event, the two titrants do yield overlapping error bands for the van’t Hoff estimates of  $\Delta H$ , as shown in Fig. 6, where they are compared with the closer of the two sets of calorimetric values. To be sure that the apparent inconsistency is not a function of our choice of fit model, we have also repeated the van’t Hoff analysis for the other two fit models, and have done both weighted and unweighted fits of the  $K$  values to Eq. (5). The approximate extreme results were obtained from weighted and unweighted vH fits of the  $K$  values from the constant-error model, as shown for  $\text{Ba}(\text{NO}_3)_2$  in Fig. 7. For none of the resulting sets of  $\Delta H_{\text{vH}}$  were more than a few of the  $\Delta H_{\text{cal}}$  points within  $2\sigma$  of the vH curve. We conclude that our calorimetric and van’t Hoff estimates of  $\Delta H$  for the  $\text{Ba}^{2+}$ /crown ether complexation are statistically inconsistent, in agreement with Liu and Sturtevant [10], however, our differences are smaller than theirs (Fig. 2).

It is worth commenting on the effect of the thermal expansivity term in Eq. (1a). If the appropriate correction term is included in Eq. (5), the maximum effect is a 1.7% increase in  $\Delta H_{\text{vH}}$  at the high- $t$  ends of Figs. 6 and 7. But the net effect of correcting the raw  $K$  values for the density variation of water and then including this thermal expansivity correction is the same as just estimating  $\Delta H_{\text{vH}}$  from the uncorrected  $K$

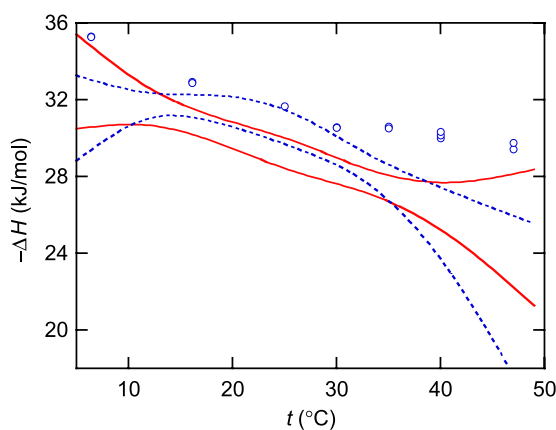


Fig. 7. van’t Hoff results for  $\text{Ba}(\text{NO}_3)_2$ , from  $K$  values estimated with the constant-error model. The solid curves represent the 1- $\sigma$  error band from the weighted vH fit, while the dashed are the same from the unweighted vH fit.

values. This makes sense, because without the density correction, for these very dilute solutions, the raw  $K$  values become effectively  $K_m$  estimates, i.e.  $K$ s based on molality reference states; and no such corrections arise in the van't Hoff analysis of  $K_m^\circ$  [21].

### 3.2. Possible causes

Most previous attempts to explain vH/calorimetric discrepancies have focused on the van't Hoff analysis, and especially the role of activity coefficients in the thermodynamic  $K^\circ$ . This approach has not been very fruitful for the present reaction (2), because even though the ionic species should have activity coefficients significantly different from 1.0, there is no reason to expect them to differ much in their  $T$  dependence. Also, since both the cation and the complex have the same charge, there is significant cancellation in the equilibrium expression,

$$K^\circ = \frac{\gamma_+(\text{complex})[\text{complex}]/c^\circ}{\gamma_+(\text{Ba}^{2+})([\text{Ba}^{2+}]/c^\circ)\gamma(\text{ether})([\text{ether}]/c^\circ)}, \quad (11)$$

where  $c^\circ$  is the unit of concentration, mol/l [21]. (The activity coefficient for the uncharged crown ether should differ insignificantly from unity at the typical concentrations of these experiments.)

Early on, we suspected that the problem might lie as much with the calorimetric  $\Delta H$  values or with the standard procedures themselves, and we chose to work with two sources of  $\text{Ba}^{2+}$  to help explore this possibility. As Fig. 8 shows, the chloride and nitrate of barium exhibit quite different heat of dilution behaviors: The chloride dilution is exothermic at all temperatures in the range of interest and at all starting concentrations, while the nitrate dilution is exothermic above  $\sim 35^\circ\text{C}$  and endothermic below and can even go from exothermic to endothermic with increasing concentration within a given dilution run (blank). Also, the magnitude of the dilution heat is significantly greater for the chloride. Thus, we hoped the results for  $\text{Ba}(\text{NO}_3)_2$  might be closer to the desired infinite dilution limit where  $\Delta H_{\text{cal}}$  and  $\Delta H_{\text{vH}}$  must agree. However, while our results for  $\text{Ba}(\text{NO}_3)_2$  are more nearly consistent than those for  $\text{BaCl}_2$ , the discrepancy remains.

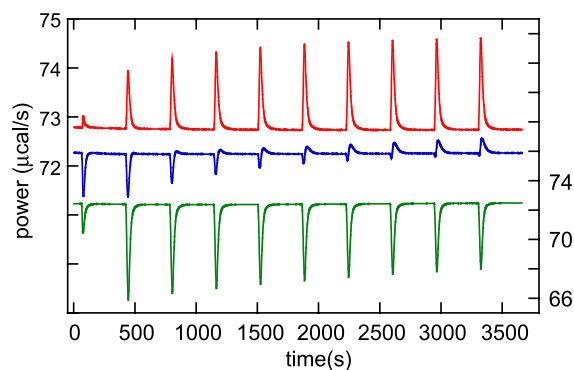


Fig. 8. Thermograms recorded for injection of 60.2 mM  $\text{Ba}(\text{NO}_3)_2$  into water at  $30^\circ\text{C}$  (top) and  $40^\circ\text{C}$  (middle), and of 54.1 mM  $\text{BaCl}_2$  into water at  $40^\circ\text{C}$  (bottom, scale to right). For  $\text{Ba}(\text{NO}_3)_2$ , the injection volumes were 4  $\mu\text{l}$  for the first and 7  $\mu\text{l}$  thereafter; for  $\text{BaCl}_2$ , 2  $\mu\text{l}$  first and 8  $\mu\text{l}$  thereafter.

Reflection on the standard procedure of subtracting a blank from the sample thermogram shows that there is an ‘apples-vs.-oranges’ component to this operation. For illustration purposes, suppose that reaction (2) has a very large  $K$  [M] $_0$ , say  $>1000$ . For early injections of titrant, the reaction goes nearly to completion, so each successive injection is into a system that has almost no  $\text{Ba}^{2+}$ . At the same time the concentrations of anion ( $\text{Cl}^-$  or  $\text{NO}_3^-$ ) and complex are building up. Past the equivalence point,  $\text{Ba}^{2+}$  begins to grow, while the concentration of complex varies little. But in the blank, the cell concentration of titrant is continually increasing. Thus, simply subtracting the blank does not properly account for the more complex processes that are occurring. In particular, it does not convert the data to the form implied by the thermodynamics: reactants at infinite dilution to products at infinite dilution. For reference, it is useful to note that for  $\text{BaCl}_2$ , the blank correction can be  $\sim 10\%$  of the largest sample peak, and it can account for most of the raw heat in late injections.

The different dilution enthalpy properties of  $\text{BaCl}_2$  and  $\text{Ba}(\text{NO}_3)_2$  are likely responsible for the different responses to change in fit model illustrated in Fig. 3. The proportional error model tends to weight the late injections more and the early ones less than the constant error model. The blank subtraction procedure should introduce different systematic errors for the two titrants, which then are manifested as different



directional changes in the estimated parameters when the fit model is changed.

There is another possible problem with the data analysis in the present case. Cations like  $\text{Ba}^{2+}$  associate with their counterions in aqueous solutions to form ion pairs, with typical  $K$  values of 5 l/mol for 2:1 electrolytes [21]. This is too small to significantly affect the stoichiometry bookkeeping under most conditions of the present study, but it might be a minor contributor to systematic error under some conditions, particularly for late injections and for small  $K$  [ether]<sub>0</sub>. However, it is not necessary to specifically consider the ion-pair formation in correcting for dissolution heat, as the phenomenological treatment of heat of dilution already includes such effects.

None of the problems considered here can be addressed through any sort of after-the-fact correction of results obtained by analyzing ITC data with the standard fit algorithms. Rather, they require alteration of the algorithm to deal specifically with the changes that occur with each injection of titrant. We are continuing work on this problem.

#### 4. Conclusion

We have used ITC to reexamine the 1:1 complexation of  $\text{Ba}^{2+}$  with 18-crown-6 ether, with the aim of better quantifying previously reported discrepancies between calorimetric and van't Hoff estimates of the reaction enthalpy. Statistically significant difference are observed not only in the calorimetric/vH comparison but also in the directly estimated  $\Delta H_{\text{cal}}$  values for our two different sources of  $\text{Ba}^{2+}$ ,  $\text{BaCl}_2$  and  $\text{Ba}(\text{NO}_3)_2$ . The discrepancies are tentatively attributed to flaws in the standard procedure, in which a blank obtained for pure titrant is subtracted from the thermogram obtained for the sample. Correction for these effects will require specific consideration of heats of dilution of not only the titrant ( $\text{BaCl}_2$  or  $\text{Ba}(\text{NO}_3)_2$ ) but also the product ( $\text{Ba}^{2+} \cdot \text{ether X}^-$ , with  $\text{X}^- = \text{Cl}^-$  or  $\text{NO}_3^-$ ). Moreover, these processes must be addressed within the fit model, as their roles change during the course of the titration.

The analysis of the ITC data was carried out using three different non-linear least-squares fit models based upon different assumptions about the data error:

constant, proportional and correlated. The statistics favor the proportional error model as the closest to reality, and we have reported results for this model. However, none of these limiting models can be correct even for all data within a given run, since very low  $q_i$  values must always be subject to the constant-error limitation, and very large are probably limited by proportional error. In general, constant and proportional error represent the extremes in experimental work, and depending on the nature of the instrumentation, one might also expect a counting-based error intermediate between these extremes, as occurs in spectrophotometry [28]. More work will be needed to properly characterize the statistical error in ITC under a variety of circumstances.

The  $n$  values obtained in the analysis of ITC data are normally ignored when the stoichiometry of the binding process is known in advance. As has been noted, this quantity represents a correction factor for the concentration of the titrate (ether) in the present experiments. Thus it is of some concern to us that most of the  $n$  values in Table 1 are significantly below the expected value of 0.99 based on our 18-crown-6 stated purity. It is possible that the shortfall stems from the deficiencies in the fit model. However, we believe that there is also an ambiguity in the stated active volume of the cell and are looking further into this possibility.

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