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Notes & Tips

Volume errors in isothermal titration calorimetry

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In the method of isothermal titration calorimetry (ITC),[1] the heat from a chemical reaction is measured for sequential injections of one reagent (the titrant) into a reaction vessel containing the other reagent (the titrate). ITC data for the simplest kinds of complexation reactions are typically analyzed by nonlinear least squares to obtain three parameters—the enthalpy change ΔH, the equilibrium constant K, and a third quantity, usually labeled n and called the "site parameter" [1–3]. In cases where the reaction stoichiometry is well understood, n serves just to correct for errors in the concentrations of the prepared solutions [4,5]. It is usually applied to the titrate, since this is often a macromolecular substance harder to prepare to known concentrations than the titrant, for which the concentration is thus tacitly taken as exactly known. For the simplest 1:1 binding processes, solutions prepared properly from pure reagents should yield $n = 1.00$.

Recently L. S. Mizoue and I [6] carried out a detailed study of the complexation of Ba^{2+} with 18-crown-6 ether in an attempt to better understand reported discrepancies between the directly estimated (calorimetric) values of ΔH and those derived from the T-dependence of K (van't Hoff) [7–9]. We were somewhat surprised to observe values of n systematically below the expected value of 0.99, based on the stated purity of our crown ether (see Fig. 1). Our experiments were done with a Model VP-ITC instrument from MicroCal, and on checking with the manufacturer I learned that the stated cell volume does not allow for displacement by the shaft and stirring paddle of the syringe assembly that is inserted into the cell when experiments are run. This means that the functional cell volume is actually smaller than that stated by the amount of the displacement. This volume error leads to the observed systematic error in n and to a similar error in the estimated K (see below).

To estimate the displacement volume I have used two independent approaches: (1) reanalysis of the ITC data for this reaction with n fixed at 0.99 but with an adjustable parameter for the cell volume V_0,[2] (2) physical measurements of the syringe shaft and stirring paddle. V_0 and n play similar roles in the fit models, since either can be used to scale the reaction stoichiometry; thus they are obtained with nearly identical relative precisions in the two fit models. The results were $V_0 = 1.372(4)\,mL$ for the $BaCl_2$ data and $1.368(3)\,mL$ for $Ba(NO_3)_2$, giving a displacement volume of $0.041\,mL$. The stirring paddle is a twisted rectangular parallelopiped roughly $16 \times 2.5 \times 0.7\,mm$, giving $0.028\,mL$. The shaft diameter is $1.27\,mm$, but its length in the active region of the cell is hard to determine precisely. I have estimated $15\,mm$, giving $0.019\,mL$ and a total displacement volume of $0.047\,mL$, which agrees reasonably well with the first estimate.

Thus the active cell volume V_0 is 3% smaller than the stated value, and correcting it gives a nearly identical percentage increase in the site parameter n. There is very little effect on the estimated ΔH values, because the measurement of q is not dependent upon assumptions about the cell volume, while the computation of ΔH is closely

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[1] *Abbreviation used:* ITC, isothermal titration calorimetry.

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[2] Since completing this work, I have learned that the other major manufacturer of ITC instruments, Calorimetry Sciences Corp., recommends a V_0 calibration procedure that is essentially this approach, down to the use of the same Ba^{2+}/crown ether complexation reaction. However, in place of direct fitting, they use trial-and-error adjustment of V_0 until n reaches its true value.
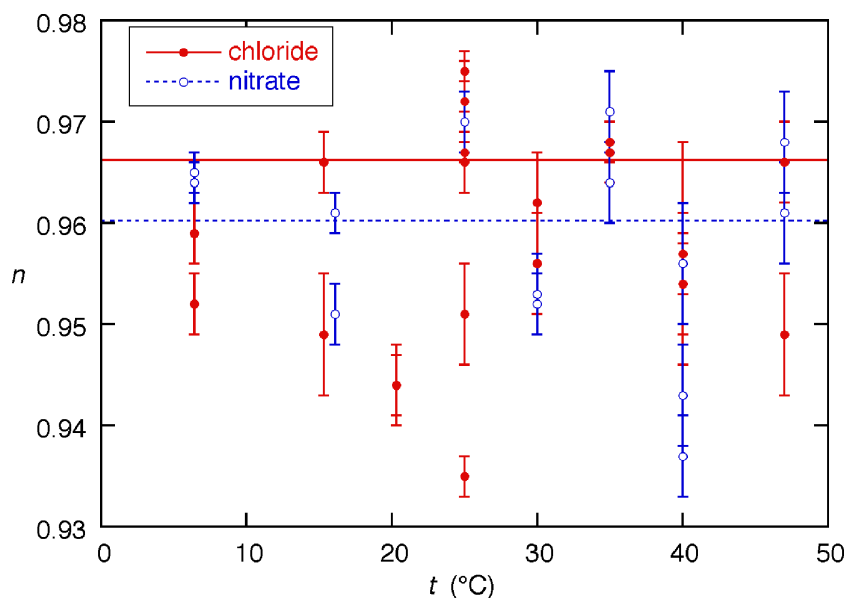
Fig. 1. Site number n from weighted (proportional error model) analysis of Ba^{2+}/crown ether complexation data from [6], using stated cell volume, $V_0 = 1.4106\,mL$. The experiments were run for two Ba^{2+} salts at several temperatures, used as the abscissa for this plot. The weighted average values and their standard errors are 0.966(2) for $BaCl_2$ and 0.960(2) for $Ba(NO_3)_2$.

tied to the delivered volume of titrant. However, there is an effect on K: Correcting V_0 by -3% and refitting the data (with n adjustable) leads to a similar 3% decrease in K. Since this correction factor is independent of temperature, it cannot significantly affect the calorimetric–van't Hoff discrepancies [6].

The VP-ITC instrument is of the perfusion type, in which solution in the active volume of the cell is expelled with each injection of titrant. It is normally assumed that the expelled solution no longer contributes to either the measured heat or the reaction stoichiometry. In the course of characterizing the displacement volume error, I have also tried analyzing data under the other extreme assumption—that all material contributes cumulatively to the heat and the stoichiometry. The results overwhelmingly support the original perfusion model, though it is possible that there remain some subtle effects relating to mixing in the boundary region.

In summary, use of the stated cell volume V_0 for the VP-ITC instrument leads to a 3% shortfall in n and a concomitant 3% excess in K for 1:1 binding processes. These errors are too small to be important in many applications of the ITC technique. However, they are systematic, so they warrant correction. And in precision work they can easily exceed the apparent statistical errors in these quantities. From the two determinations, my best estimate of the displacement volume is 0.044 mL. With allowance for uncertainties in the crown ether purity and in the physical measurements, this value is likely within 0.01 mL of the true value.

Acknowledgments

References

[1] T. Wiseman, S. Williston, J.F. Brandts, L.-N. Lin, Rapid measurement of binding constants and heats of binding using a new titration calorimeter, Anal. Biochem. 179 (1989) 131–137.

[2] M. El Harrous, S.J. Gill, A. Parody-Morreale, Description of a new Gill titration calorimeter for the study of biochemical reactions. I: assembly and basic response of the instrument, Meas. Sci. Technol. 5 (1994) 1065–1070.

[3] M. El Harrous, O.L. Mayorga, A. Parody-Morreale, Description of a new Gill titration calorimeter for the study of biochemical reactions. II: operational characterization of the instrument, Meas. Sci. Technol. 5 (1994) 1071–1077.

[4] J. Tellinghuisen, A study of statistical error in isothermal titration calorimetry, Anal. Biochem. 321 (2003) 79–88.

[5] J. Tellinghuisen, Statistical error in isothermal titration calorimetry, Methods Enzymol. 383 (2004) 245–282.

[6] L.S. Mizoue, J. Tellinghuisen, Calorimetric vs. van't Hoff binding enthalpies from isothermal titration calorimetry: Ba^{2+}-crown ether complexation, Biophys. Chem. 110 (2004) 15–24.

[7] Y. Liu, J.M. Sturtevant, Significant discrepancies between van't Hoff and calorimetric enthalpies. II, Protein Sci. 4 (1995) 121–126.

[8] J.B. Chaires, Possible origin of differences between van't Hoff and calorimetric enthalpy estimates, Biophys. Chem. 64 (1997) 15–23.

[9] J.R. Horn, D. Russell, E.A. Lewis, K.P. Murphy, van't Hoff and calorimetric enthalpies from isothermal titration calorimetry: are there significant discrepancies? Biochemistry 40 (2001) 1774–1778.