Article

# Isothermal Titration Calorimetry Can Provide Critical Thinking Opportunities

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S Supporting Information

ABSTRACT: College chemistry faculties might not have considered including isothermal titration calorimetry (ITC) in their majors' curriculum because experimental data from this instrumental method are often analyzed via automation (software). However, the software-based data analysis can be replaced with a spreadsheet-based analysis that is readily accessible to undergraduate students. This article describes a three-phase study (three lab periods) of cation–hexametaphosphate complexation via ITC, including the asymptote method by which students perform their own ITC data analysis, to demonstrate how ITC experiments can provide students with critical thinking opportunities comparable to other data-analysis-intensive physical-analytical experiments.

KEYWORDS: Calorimetry/Thermochemistry, Coordination Compounds, Curriculum, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Laboratory Instruction, Physical Chemistry, Thermodynamics, Upper-Division Undergraduate
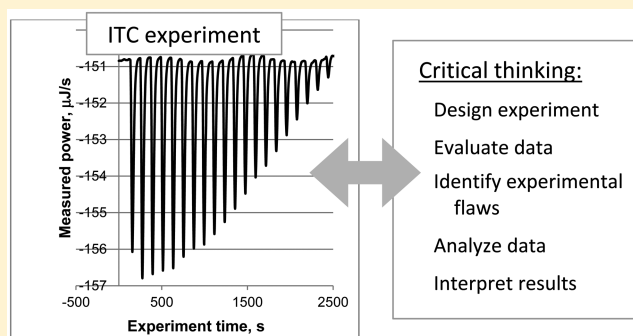
■ INTRODUCTION

Chemistry educators traditionally require that chemistry students perform experiments in which they collect and evaluate complex sets of numerical data. This is done, at least in part, in order to give students experiences through which they might practice critical thinking about their scientific observations. Because isothermal titration calorimetry (ITC) data are usually analyzed via software, many college chemistry departments might not have considered curricular implementation of ITC as a potential critical thinking opportunity. However, it is rather straightforward for students to learn to use spreadsheets to calculate thermodynamic parameters from ITC data. Performance of the ITC experiment then includes experimental design, qualitative evaluation of data, identification of experimental flaws, quantitative analysis of data, and interpretation of results. These certainly fall under the definitions of critical thinking examined by Tsai[1] and are related to the ability to "draw appropriate conclusions" cited in *ACS Guidelines and Evaluation Procedures for Bachelor's Degree Programs* as a target skill for chemistry majors.[2]

In an ITC experiment one reactant is titrated into a sample cell containing a second reactant. During the experiment, the ITC instrument maintains the sample cell and an adjacent reference cell at the same temperature and records, over time, the power required to do so. Spikes in that power signal correspond to heat evolution or absorption associated with chemical reaction(s) occurring in the sample cell upon each titrant addition. Analysis of ITC data provides scientists with full thermodynamic characterization (ΔH_R, ΔG_R, ΔS_R, and K_{eq}) of chemical reactions from single experiments. As a result of

this utility, the technique has been gaining prominence in the chemistry and biochemistry research literature. As of this writing, over 6400 research articles in the ACS journals database (pubs.acs.org) include the phrase "isothermal titration calorimetry" or just "ITC", and over 3900 of those were published in the past decade. Many of these articles describe studies of ligand-to-biomolecule binding, and commercial ITC instruments for microscale experiments have made this type of experiment routine in well-equipped biochemistry laboratories.[3] However, more and more of the latest ITC-based research articles are not confined to biochemistry as ITC is now frequently implemented to study nonbiological binding types. Very recent examples of nonbiological ITC applications in the chemistry literature include quantification of ion exchange in mineral suspension,[4] systematic survey of small-molecule Lewis acid–base adduct formation,[5] and study of ligand binding to quantum dots.[6]

When the Department of Chemistry at Mercer University recently overhauled the extended-projects-based laboratory courses for junior-level chemistry majors, the faculty decided to deploy ITC as a core method featured in these courses. The department was fortunate to receive a National Science Foundation grant under the Transforming Undergraduate Education in Science, Technology, Engineering, and Mathematics (TUES) program to assist with the purchase of a microscale ITC instrument. This article describes one of the ITC experiments implemented in the extended-projects-based lab course, a binding titration experiment, including how

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students were asked to work directly with their data, to think critically, and to calculate their own results rather than relying on automated data analysis.

While this article has been aimed primarily at chemistry and biochemistry faculties considering the introduction of ITC into their undergraduate curriculum, there is also some information about the ITC experiment in here to share with *JCE* readers in departments without the instrumentation. The ITC data and the instructor notes about the spreadsheet-based ITC data analysis method included in the Supporting Information might be especially useful for readers who want to give students practice working with ITC data. Whatever their departments' current situations with respect to ITC instrumentation, some readers might want to compare the general approach and critical thinking opportunities described here with their physical-analytical lab curriculum and the instrumental methods their students use. Readers considering the curricular implementation of ITC might also look at previous *JCE* articles that described the construction of a (less expensive) benchtop instrument for macro-scale titration calorimetry measurements and some instructional experiments with that instrument.[7,8]

■ OVERVIEW OF THE EXTENDED PROJECTS BASED LAB COURSE

The Department of Chemistry at Mercer University has replaced separate junior-level laboratory courses (covering analytical, physical, and inorganic chemistry) for chemistry majors with a two-course sequence called Problems in Chemistry. In these courses, students spend 6 h per week in the laboratory working on a series of four extended projects. Each project lasts one-half of a semester, and students write full reports (ACS article style) on their progress three times per project. The specifics of the projects vary from year to year, but the goal has always been to give chemistry majors lab experiences in which they integrate concepts from the various chemistry subdisciplines. The extended-project-based lab courses were implemented in order to provide a transitional bridge between the traditional series of discrete one-week lab experiments in the first two years' chemistry courses and the open-ended research projects that engage most of our chemistry majors throughout their senior year. Other pedagogical benefits of incorporating research-type experiences into the curriculum are well established, including increased interest, increased confidence, increased understanding, and increased ability.[9]

Student feedback and anecdotal observations have suggested that the Problems in Chemistry students more readily practiced critical thinking when there were scientific connections between the projects they worked on. Therefore, the recent overhaul of these lab courses included the more intentional implementation of themes or synergies, especially shared methods, cross-project collaborations, and chemical similarities between projects. One such connection was the deployment of ITC in three of the four projects. For example, a project sequence from the 2014−2015 offering of the Problems in Chemistry courses is outlined in Table 1. In each case, the ITC experiment was only one part of the total project. The students' return to ITC again and again after their initial introduction led to their increased familiarity with the experiment and enabled progression to more complex applications requiring more critical thinking.

This article describes the progress of the major ITC component of the cadmium sulfide quantum dots project

Table 1. Problems in Chemistry Projects (2014−2015)

Project Description:	ITC Used:
(1) Developing scientific writing skills with a fermentation experiment	No ITC
(2) Synthesis and exploration of gold nanoparticles	Introduction to ITC with binding titration; analysis of data via software
(3) Proposal of cadmium sulfide quantum dot formation mechanism	Return to ITC with binding titration; analysis of data via spreadsheet (see calculating thermodynamic parameters section below)
(4) Verification of caspace-3 cellular substrates	Return to ITC again with more complex experiment (enzyme kinetics assay); analysis of data via spreadsheet

(project no. 3 in Table 1) as an example of how an ITC experiment can provide opportunities for critical thinking. The whole 14-lab-period project aimed students at developing a physical model to predict the number and size of quantum dots that form in the arrested precipitation of cadmium sulfide in the presence of sodium hexametaphosphate. As one part of the series of experiments leading up to that goal, students studied the initial complexation of cadmium(II) cations to hexametaphosphate anions via ITC binding titration. Instruction included running the ITC experiments and associated experiments, as well as the data analysis method based on constructing spreadsheets (see Calculating Thermodynamic Parameters section). Readers interested in other instructionally deployed ITC experiments might look at a recent laboratory experiment in this *Journal* describing the gold nanoparticles project from our courses (project no. 2 in Table 1).[10]

■ PROGRESS OF AN ITC BINDING TITRATION EXPERIMENT

The association of divalent cations with sodium hexametaphosphate to produce complex ions through a form of ion exchange has previously been studied via polarography,[11] selective-electrode potentiometry,[12] quantitative redox titration,[13] and electron spin resonance (ESR) spectroscopy.[14] In the extended-project-based laboratory course described in the preceding section, chemistry majors have used ITC to characterize this type of association for cadmium(II) cations:

$$Cd^{2+}(aq) + Na_6(PO_3)_6(aq)$$
$$\rightarrow 2Na^+(aq) + CdNa_4(PO_3)_6(aq) \qquad (1)$$
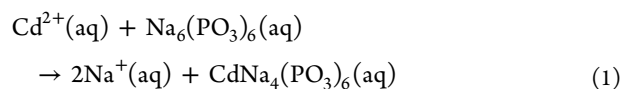
In a typical experiment, students titrated 1.25 mL of 0.250 mM cadmium(II) nitrate with 20 (10-μL) aliquots of 1.00 mM sodium hexametaphosphate, while the sample cell was maintained at constant 25.0 °C. In one such experiment, the resulting net endothermic reaction yielded the data shown in Figure 1.

To obtain ITC data from which an experimenter might fully characterize the thermodynamics of the association, whether covalent or noncovalent, between some molecular couple, a solution of one member of the couple is titrated, typically in identical aliquots, into a solution of the other member. As can be seen in Figure 1, the resulting heat starts out larger in magnitude for initial additions and then drops precipitously when the experiment passes through the molar equivalence point in the same fashion as in a pH titration curve. Finally, the per-aliquot heat dwindles toward some consistent amount that results from small contributions like heat of dilution, changes in heat capacity, and/or persistent secondary reactions. The progressively decreasing heat corresponds to the relative extent
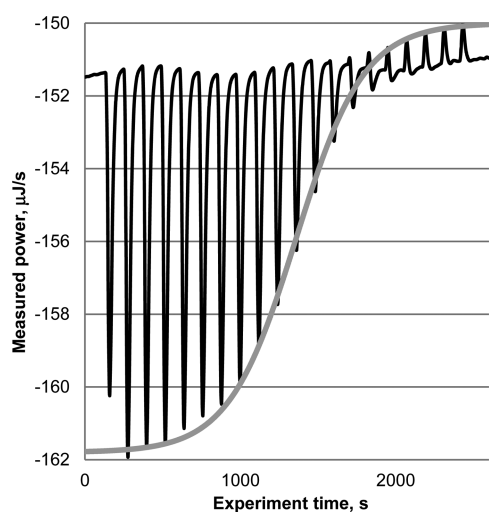
Figure 1. Raw power vs time data for the titration of 0.250 mM cadmium(II) nitrate with 1.00 mM sodium hexametaphosphate. The superimposed sigmoidal curve highlights the titration curve shape. The first aliquot's peak is typically depleted due to a small amount of pre-experiment reaction while the instrument thermally equilibrates.

of reaction similarly decreasing over the course of the experiment. The ITC instrument actually measures the power applied to maintaining the sample and reference cells at the same temperature over time, and then experimenters apply software integration to convert those data, the peaks in Figure 1, into heat per mole of titrant for each addition, the plot of which (called the binding isotherm) resembles the familiar titration curve as shown in Figure 2.
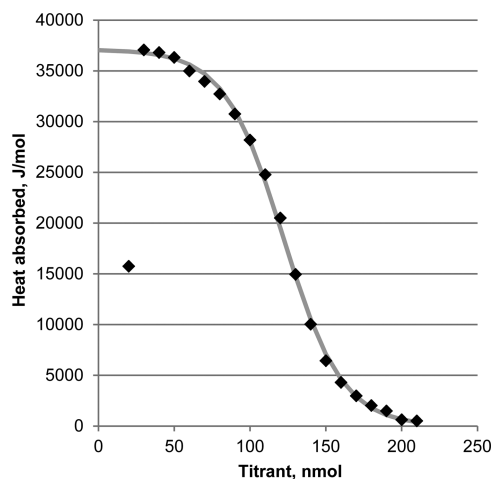

Figure 2. Heat absorbed per mole of titrant added over the course of the titration of 0.250 mM cadmium(II) nitrate with 1.00 mM sodium hexametaphosphate (same experiment as Figure 1). The superimposed sigmoidal curve can be used to determine the enthalpy of reaction (asymptote method). As in Figure 1, the first aliquot's data point exhibits the typical depletion.

Since the heat measured in an experiment might include primary binding plus any secondary system changes (heats of dilution, conformational changes, other reactions of the species under investigation, impurity reactions, etc.), the ITC technique is somewhat limited and might require control experiments (e.g., titrating one component into solvent only) in order to separate the heat due to the primary binding from the

total heat measured.[15] Only careful design of the ITC experiment ensures that the resulting data fall within the practical range of the method: If experimental concentrations are too high, the resulting titration curve will be too precipitous for productive data analysis. (See the Calculating Thermodynamic Parameters section below.) And if concentrations are too low, the resulting heat due to primary binding will be too small for reliable measurement. ITC experts[15] recommend selecting total analyte concentration $[A]_T$ within a range defined by this inequality:

$$1 < K_{ac}[A]_T < 1000 \tag{2}$$

in which K_{ac} is the association constant, a concentration-based equilibrium constant for 1:1 binding of titrant T to analyte A, forming product AT:

$$K_{ac} = \frac{[AT]}{[A][T]} \tag{3}$$

Software for analyzing ITC data, like TA Instruments' NanoAnalyze,[16] can also be used as an experimental design tool by deploying a predictive mode in the software to model what experimental results might look like for a binding with known (or approximate) values for enthalpy of reaction and association constant.

The thermodynamic study of the interaction described by eq 1 was distributed over three lab periods.

Period 1: Preliminary Experiment

The students' design of an ITC experiment was first informed (before running that experiment) with a separate preliminary experiment: a fluorometric titration. On the basis of the observation that this complexation is accompanied by increasing intensity of a fluorescence peak at 405 nm, shown in the inset of Figure 3, students designed an experiment to determine K_{ac} for 1:1 binding by treating Cd^{2+} as the ligand, titrating it into a solution of hexametaphosphate, and monitoring the resulting fluorescence intensity. This method
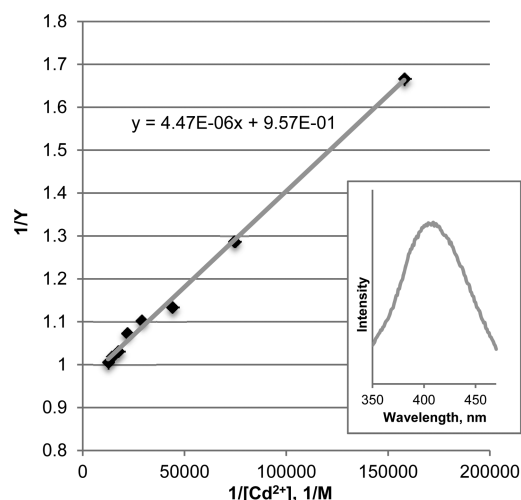

Figure 3. Hughes−Klotz plot (reciprocal fractional saturation vs reciprocal ligand concentration) for a fluorometric titration experiment in which cadmium(II) nitrate was added in aliquots to a solution of sodium hexametaphosphate. The reciprocal of the slope approximates the association constant for 1:1 binding (2.2×10^5 M^{-1} in this case). The inset shows the fluorescence peak that was monitored over the course of this experiment.

recasts the ion-exchange type of chemical reaction eq (eq 1) as a 1:1 ligand binding:

$$\text{free } Cd^{2+} + \text{available phosphate site} \rightarrow \text{complexed } Cd^{2+} \tag{4}$$
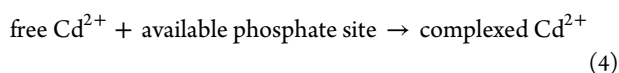
Students continued the fluorometric titration experiment until the fluorescence intensity maximized. Then, they approximated fractional saturation (Y) of the hexametaphosphate for all additions based on the assumption that relative signal (fluorescence intensity increase) was directly proportional to relative extent of reaction and on calculation of concentrations for all three components in the 1:1 binding reaction above (eq 4):

$$Y = \frac{[\text{occupied phosphate sites}]}{[\text{total phosphate sites}]} = \frac{[Cd(PO_3)_6{}^{4-}]}{n[(PO_3)_6{}^{6-}]_T} \tag{5}$$

where $[Cd(PO_3)_6{}^{4-}]$ is the total concentration of cadmium-to-phosphate complexations and $n[(PO_3)_6{}^{6-}]_T$ is the total hexametaphosphate concentration times the maximum number of cadmium cations that can coordinate per hexametaphosphate molecule. The total number of phosphate sites was subsequently determined to equal about three times the number of hexametaphosphate molecules ($n = 3$ above) from the position of the equivalence point in the ITC binding titration experiment, the inflection point of the superimposed sigmoidal curve in Figure 2. So the complete chemical reaction in this case is more accurately written:

$$3Cd^{2+}(aq) + Na_6(PO_3)_6(aq)$$
$$\rightarrow 6Na^+(aq) + Cd_3(PO_3)_6(aq) \tag{6}$$
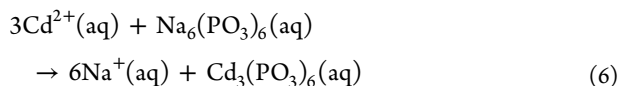
Instructors have the options of either revealing the ~3:1 stoichiometry in advance of experimentation or allowing students to discover this on their own, typically from an ITC experiment during the second period. (For more information, please see the instructor guide in the Supporting Information.)

With fractional saturation and remaining concentration of Cd^{2+} both determined for each addition, students constructed a Hughes−Klotz type of plot,[17] a linear fit to the double-reciprocal form of the noncooperative Hill equation, also known as the Langmuir isotherm:

$$\frac{1}{Y} = \frac{1}{K_{ac}[L]} + 1 \tag{7}$$

in which $[L]$ is the ligand concentration, $[Cd^{2+}]$ in this experiment. An example Hughes−Klotz plot for this experiment is shown in Figure 3. The association constant (K_{ac}) for noncooperative 1:1 binding treating each cadmium(II)−phosphate interaction as independent, as represented above by eq 4, is the reciprocal of this plot's slope. A trace of the points in Figure 3 does exhibit slight curvature, rather than a perfect straight-line fit, possibly indicating that the sequential complexations of Cd^{2+} cations to the hexametaphosphate are not entirely independent (i.e., apparent ΔG_R changing slightly over the course of the experiment). Students were not asked to analyze these results any further, but they did return to them when interpreting the results of the ITC binding titration. Table 2 includes, among other results, a fluorometry-based 1:1 binding K_{ac} value from the Spring 2015 offering of the extended-projects-based course. There is an example setup of the fluorometric titration data analysis in a Microsoft Excel spreadsheet included in the Supporting Information.

Table 2. Set of Student Results (Spring 2015)

Result	Fluorometric	ITC 1[a]	ITC 2[a]	Software
K_{ac} (M^{-1})	2.2×10^5	3.8×10^5	2.5×10^5	7.4×10^5
ΔH_R (kJ/mol)	N/A	38	37	34

[a]ITC 1 results were calculated by the asymptote method from the impurity-assumption-corrected data from an experiment in deionized water (Figure 4); ITC 2 results were calculated by the asymptote method from data from an experiment performed in ultrapure water (Figure 2).

Period 2: Initial ITC Experiment(s)

On the basis of their preliminary fluorometric titration experiment, students were able to design their ITC experiment by scaling down (to fall within the range described by eq 2) and adjusting concentrations to replicate (across the range of aliquot additions in the ITC) approximately the fractional saturations they had previously observed via fluorometry. (See the instructor guide in the Supporting Information for more details.) The heats (per mole of titrant) determined for each addition in this first ITC experiment are shown in Figure 4. An
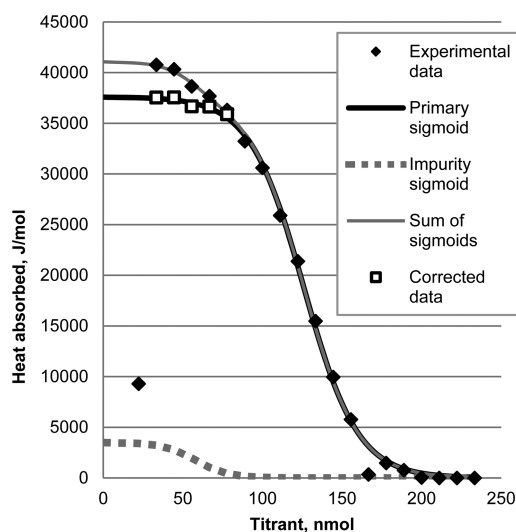

Figure 4. Heat absorbed per mole of titrant added over the course of a titration of 0.250 mM cadmium(II) nitrate with 1.00 mM sodium hexametaphosphate (filled diamond data points) in deionized water rather than ultrapure water (shown in Figure 2). The figure also shows the two sigmoidal curves used to fit the measured data and the impurity-reaction-adjusted data (open square data points) used to calculate K_{ac}.

initial attempt to analyze these data using the asymptote method (described in the Calculating Thermodynamic Parameters section below) yielded very inconsistent results with the association constant apparently varying dramatically over the course of the experiment.

When prompted to suggest a cause for this inconsistency, students pointed to the odd shape of the titration curve, even eventually proposing some reaction involving an impurity as culprit. They proceeded to fit a sum of two sigmoidal curves to the data and then subtracted the secondary (much smaller) contributing curve from the measured heats to yield a corrected data set, also represented in Figure 4. Application of the asymptote method to the corrected data set resulted in determination of an enthalpy of reaction (ΔH_R) and a consistent K_{ac} value for 1:1 binding similar to that found

from the Hughes–Klotz analysis of the fluorometric titration experiment (both results also included in Table 2).

Period 3: Follow-Up ITC Experiment(s)

To determine whether that extra heat contribution in the first titration of cadmium(II) with sodium hexametaphosphate (Figure 4) was due to some impurity reaction, the experiment was repeated but with ultrapure water replacing deionized water in the solution preparations. (See the instructor guide in the Supporting Information for more details.) The data from an ITC binding titration in ultrapure water were used to create Figures 1 and 2, and comparison of Figure 4 with Figure 2 shows that the switch to ultrapure water effectively removed that extra heat contribution. Application of the asymptote method (described below) to these data yielded results (ΔH_R, K_{ac}) very similar to the 1:1 binding K_{ac} from the fluorometric titration, very similar to the ΔH_R from the previous ITC experiment (with the corrected data set), and similar in magnitude to the K_{ac} from that same previous ITC experiment. These results are included in Table 2.

For comparison, the same data from the titration of cadmium(II) nitrate with sodium hexametaphosphate in ultrapure water (Figures 1 and 2) were also analyzed with TA Instruments' NanoAnalyze software,[16] using an independent-sites (noncooperative) 1:1 binding model. Rather than the asymptote method that the students used, software-based analysis typically employs the iterative method (also described below). The software user inputs starting parameters (ΔH_R, K_{ac}) for data fitting, and the software then goes through a number of iterations to home in on the parameters (modified from the starting values) that best fit the data. Table 2 shows slightly different values for ΔH_R and K_{ac} in the two rightmost columns, the asymptote method (students) and iterative method (software) results from the same data set. Students might notice that automated software-based analysis also seems to exhibit some dependence on initially selected values, especially if the software has been set to a small number of iterations. Software results might be verified by deploying different starting parameters and increasing the number of iterations until the software analysis yields consistent parameters (ΔH_R and K_{ac}), which was done in this case.

The range of results reported in Table 2 is representative of what students have observed for this project. With two different experimental methods (fluorometric titration and ITC), two different ITC experiments (deionized water and ultrapure water), and two different methods for ITC data analysis (asymptote method and iterative method, both described in the next section), students were initially somewhat challenged to reconcile this range of results. However, because they have spent so much time pouring over spreadsheets, analyzing data, it has not been much of a leap for students to recognize that considering their methods (including any assumptions made as part of the data analysis) is just as important as considering their results. Internalization of this notion is most likely part of developing critical thinking skills. In this case, the discrepancy between the asymptote method analysis and the iterative method analysis of the same data set (rightmost columns in Table 2) probably points to a bad assumption, to the fact that a second or third cation coordination to a hexametaphosphate is not actually independent of any previous complexation.

■ HAZARDS

Cadmium(II) nitrate is highly reactive with reducing agents, combustible materials, and organic materials. It is extremely hazardous in case of ingestion, very hazardous in case of inhalation, hazardous in case of skin contact, and a suspected carcinogen. Supervision of its use by students should include appropriate safety wear (goggles, lab coat, and gloves) and appropriate storage (sealed in a cool, well-ventilated location).

■ CALCULATING THERMODYNAMIC PARAMETERS

The accuracy of results from both analytical calculation methods described below will depend on whether the ITC instrument has been calibrated[18] and whether the raw data have been corrected (background and/or control experiment subtraction) to remove heat contributions from dilution and auxiliary chemical reactions.[15] Both methods require integration of peaks in the power over time data (like Figure 1) to yield heat per addition data (like Figure 2). Also, the following analysis method descriptions assume application to determine ΔH_R and K_{ac} for independent 1:1 binding. More complex situations, like cooperative or competitive binding of more than one titrant molecule per analyte molecule (or vice versa), would require significantly more involved model fitting that has not been covered in the Problems in Chemistry lab courses. However, ITC manufacturers' analytical software packages like TA Instruments' NanoAnalyze[16] include models for fitting data to these more complex binding scenarios.

Iterative Method

The iterative method solves for the enthalpy of reaction (ΔH_R) and association constant (K_{ac}) simultaneously by starting from an approximate association constant value and then calculating hypothetical enthalpies of reaction for each addition in the titration data set. On the basis of the variation in those calculated enthalpies, the approximate association constant value is then adjusted, and the enthalpies are recalculated. This process continues, through a number of iterations, until the selected association constant yields reaction enthalpies with the minimum possible variation among the set, usually as determined by the error square sum.[19] Because of the large number of calculation-adjustment-recalculation cycles required to home in on the final results, the iterative method is only appropriate for computer-powered data analysis and not manual (via spreadsheet) analysis by students (or by scientists). ITC manufacturers' analytical software packages like TA Instruments' NanoAnalyze[16] employ the iterative method.

Asymptote Method

The asymptote method involves first determining the enthalpy of reaction from analysis of the ITC data set, and then subsequent calculation of the net extents of reaction and the corresponding association constant values at each aliquot addition. So this method is, in some respects, the opposite of the iterative method. Because the asymptote method can be executed (and repeated) easily with a spreadsheet, it is an appropriate way for students to tackle their ITC data and calculate their own results (rather than relying on software analysis). The introduction of this method in the Problems in Chemistry courses has begun with a 10 min lecture describing the method, and then continued with each student constructing a spreadsheet (in a computer lab) while faculty members circulated, answering questions and offering advice. An example

spreadsheet (same data as Figure 2) with explanatory annotations is included as Supporting Information.

In the asymptote method, the enthalpy of reaction (ΔH_R) is simply determined from the nonzero asymptote of a sigmoidal curve fit to the plot of heat evolved per mole of titrant per addition vs total moles of titrant added (binding isotherm). Since the relative extent of reaction approaches unity as the concentration of titrant approaches zero (the complete reaction of a vanishingly small addition of titrant can be assumed), the amount of heat evolved per mole of titrant similarly approaches the enthalpy of reaction for a 1:1 binding.[20] Figures 2 and 4 both show fits of sigmoid curves to the binding isotherms. This type of fit is fairly useful for estimating enthalpy of reaction from an asymptote and can be performed with a spreadsheet and curve parameter tweaking to achieve a manual least-squares fit. The sigmoid curve equation is

$$y = A\left[1 - \frac{1}{1 + e^{B(C-x)}}\right] \tag{8}$$

in which parameter A scales the curve (sets the nonzero asymptote), parameter B determines the degree of curvature (steepness), and parameter C places the inflection point. The example spreadsheet in the Supporting Information demonstrates the least-squares fit of a sigmoidal curve to the data points in Figure 2. If the manual sigmoid fit is not appropriate, then a linear fit or polynomial fit to the initial data points in a binding isotherm can still yield a fair estimate of enthalpy of reaction from the y-intercept.

Once the enthalpy of reaction is known, the total extent of reaction at each addition is calculated from total heat evolved through to that addition relative to the total moles of titrant added through that same point. Then, a concentration-based association constant value can be calculated, for the at-equilibrium system after each addition, from total amounts of product formed and reactant remaining based on each total extent of reaction. Of course, changing total volume in the sample cell must also be included. This whole process entails pretty extensive spreadsheet usage, and the Problems in Chemistry students have typically created one template spreadsheet (the first time they went through this analysis) that they then reproduced for all subsequent analyses of data from additional ITC binding titrations.

The very high relative error resulting from very little product formed (for the earliest points in the titration) or very little reactant remaining (for the last points in the titration) means that the lowest-uncertainty association constant results will come from the middle (steeper) part of the titration curve. For students' first pass at manual (via spreadsheet) analysis of ITC data, it might be appropriate just to average those lowest-uncertainty association constants. However, there are additional considerations that might be introduced to further exercise students' critical thinking about the ITC experiment:

(a) The asymptote method assumes that the net heat (J/mol) determined from the sigmoidal curve fit corresponds to a consistent enthalpy of reaction for the net process under investigation. What if that assumption is flawed? (Why might that be the case? And what would be the effect on the asymptote method of analysis?)

(b) The iterative method assumes a consistent free energy of reaction and therefore a consistent association constant throughout the titration experiment. However, the asymptote method might produce the apparent result

of association constant continuously changing over the course of the titration. (Why might that be the case? And what would be the effect on the data analysis?)

Auxiliary Calculations

Once K_{ac} has been determined (by either method), the Gibbs free energy of reaction (ΔG_R) can be estimated with the assumption that the concentration-based equilibrium constant is approximately equal to the thermodynamic equilibrium constant (K_{eq}), a valid assumption when the activity coefficients are close to unity:

$$K_{ac} \approx K_{eq} \tag{9}$$

$$\Delta G_R = -RT \ln(K_{eq}) \tag{10}$$

where, of course, R is the gas constant (8.314 J/mol·K) and T is the temperature. Then, the entropy of reaction (ΔS_R) can finally be calculated:

$$\Delta G_R = \Delta H_R - T\Delta S_R \tag{11}$$

Thus, ITC provides a method by which complete thermodynamic characterization (ΔH_R, ΔG_R, ΔS_R, and K_{eq}) of a chemical process can be achieved from a single set of experimental data.

■ CONCLUSION

Critical thinking is not always something chemistry faculties overtly program into chemistry laboratory curricula even though it is something they hope students will develop from their laboratory and postlaboratory experiences. Because the results of ITC experiments are often interpreted solely through software-powered data analysis, some chemistry faculty might not have considered adding ITC to their majors' curriculum. However, a spreadsheet-based method with which to analyze ITC data and to calculate thermodynamic parameters as results is readily accessible to undergraduate students. The three-phase ITC experiment described here includes experimental design, qualitative evaluation of data, identification of experimental flaws, quantitative analysis of data, and interpretation of results. This example demonstrates how ITC experiments might be curricularly deployed as possible opportunities for chemistry majors to practice critical thinking, either alongside or in place of other data-analysis-intensive physical-analytical experiments.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00575.

Annotated spreadsheet (Excel) for applying asymptote method to calculating ΔH_R and K_{ac} from ITC binding titration data, and for constructing Hughes−Klotz plot (XLSX)

Instructor guide (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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