Notes & Tips

The role of backlash in the "first injection anomaly" 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 dependence of the heat q on extent of reaction constitutes a titration curve, the analysis of which yields the enthalpy change $\Delta H°$ and the equilibrium constant K for the reaction [1–3]. In ITC experiments, it is common practice to initiate each run with a small "throwaway" injection of titrant. The rationale for this procedure has been that the heat observed from this first injection is systematically smaller in magnitude than expected, and the prevailing explanation is preinjection loss of titrant through diffusive mixing of material near the tip of the syringe with the solution in the reaction vessel.

Our recent observations have led us to conclude that a primary cause of this first injection anomaly can be backlash in the motorized screw mechanism used to drive the syringe plunger. The standard procedure for conducting an ITC experiment calls for one or two "purge-and-refill" operations of the syringe just prior to installing the syringe/stirring mechanism on the instrument.[2] In this operation, the plunger is driven down and then up again, emptying and recharging the syringe with titrant from a reservoir. If this is the final operation before the start of the run, the first injection can be subject to backlash error from the change in direction of the drive screw. This effect can be a significant source of systematic error, since under the prevailing interpretation of the anomaly, the titrant is assumed to have entered the reaction mix and therefore to contribute to the

reaction stoichiometry, whereas with backlash there is a shortfall of titrant in the first injection that is never compensated in subsequent injections. Fortunately, there is a simple solution to this problem: After the final purge-and-refill operation, execute a short "down" movement of the plunger before inserting the syringe into the reaction cell.

The observations that led us to the backlash interpretation were made in conjunction with studies of the complexation of Ba^{2+} with 18-crown-6 ether [4], a reaction that has been subject to scrutiny in connection with questions about the consistency between the directly measured (calorimetric) estimates of $\Delta H°$ and those derived from the T dependence of K (van't Hoff) [5–7]. In some of the runs, we sought to save time by working with titrant concentrations large enough to permit running several experiments without refilling the syringe. This meant that some of the runs did *not* start from the purge/refill operation. The contrast with those that did was striking, as illustrated in Fig. 1. Note particularly that not only is there a shortfall of heat in the first injection but also there is a slight *excess* in all subsequent injections. There is also a temporal delay in the onset of the response to the first injection. These observations are fully consistent with expectations for the backlash interpretation.

To quantify the effect we processed our data for 14 runs with $Ba(NO_3)_2$ and for 23 runs with $BaCl_2$, using a nonlinear least-squares fit model for 1:1 complexation [8] that had been modified to include a parameter representing the titrant volume error Δv in the first injection, under the assumption that all disparity in the heat for that injection was due to such error. Results for Δv are shown in chronological order in Fig. 2. In all of the runs represented by values near the top of the plot, a "down" command was executed just before the

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[1] *Abbreviation used:* ITC, isothermal titration calorimetry.
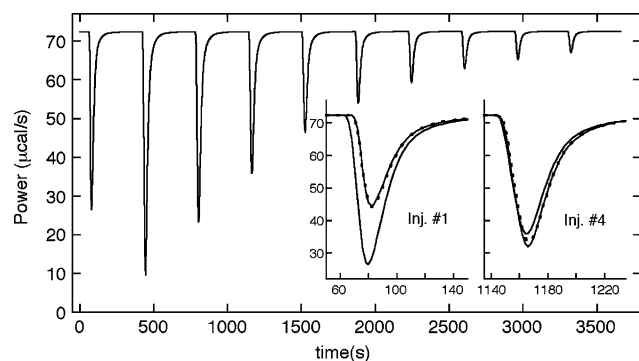[2] Instruction manual for MicroCal Model VP-ITC.

Fig. 1. ITC thermogram for titration of 1.09 mM 18-crown-6 ether with 60.2 mM $Ba(NO_3)_2$ at 40 °C, using a MicroCal VP-ITC instrument. The cell volume was 1.41 mL and the injection volumes were 4 μL for the first and 7 μL for subsequent injections. The insets show the first and fourth peaks for two runs that directly followed a purge/refill operation (overlapped solid and dashed) and one that was preceded by a "down" command.
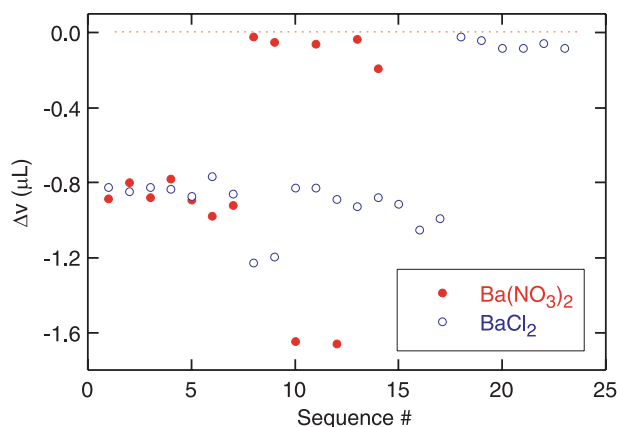

Fig. 2. Derived volume errors Δv in first injection, plotted as a function of chronological order for experiments employing both $Ba(NO_3)_2$ and $BaCl_2$ as titrant. With the exception of runs 10 and 12 for $Ba(NO_3)_2$, all of the recent runs (8–14 for $Ba(NO_3)_2$, 18–23 for $BaCl_2$) were preceded by a "down" plunger command. In contrast, all of the earlier runs followed a purge/refill operation. Most of the recent data involved titrant volumes close to those given in the caption to Fig. 1, while the earlier runs employed volumes larger by a factor of 2 or 3.

syringe was mounted on the instrument. The fitted volume shortfall exceeded 0.1 μL in only 1 of these runs. This amount thus represents a rough estimate of the error due to the traditionally invoked diffusive mixing. In the 2 purge/refill-preceded runs illustrated in Fig. 1, the shortfall was 1.65 μL. All of the early runs for both titrants were conducted in the standard mode; with the exception of runs 8 and 9 for $BaCl_2$, these showed volume shortfalls in the 0.8- to 1.0-μL range.[3]

1:1 Complexation reactions are normally modeled with three fit parameters: $\Delta H°$, K, and a parameter n

usually called the "site number" that corrects for concentration errors in the "macromolecule" (here the crown ether) [8]. For the present data, neglect of Δv has its greatest effect in the case of runs 10 and 12 for $Ba(NO_3)_2$ (illustrated in Fig. 1), for which the volume shortfall comprises ∼6% of one equivalent of titrant. Interestingly, $\Delta H°$ is almost completely immune to such errors. This is because its value is closely tied to the amount of titrant delivered to the reaction vessel in injections past the first, and in the standard fit models, the concentration of titrant is fixed at the stated value of preparation. On the other hand, neglect of Δv leads to percentage increases in K and n almost as great as the 1-equivalent error or ∼6% in the two noted cases. By contrast, when Δv is included in the fit model, the K values for the three runs in question span a range of only 1% and the n values less than 2%. The consequences of neglecting Δv are considerably smaller for the other runs, due to the smaller magnitude of the error, combined with the use of larger titrant volumes for each injection.[4]

For the 11 runs that were preceded by a "down" command, the magnitude of the effective shortfall was so small that it makes little difference to the analysis whether the shortfall is attributed to preinjection mixing or to undelivered titrant. The maximum difference between the two fit models on these runs was a 0.7% change in the estimated K and n values for the last $Ba(NO_3)_2$ run illustrated in Fig. 2. However, the effect is still systematic and large enough to require deletion of the first point in the standard analysis.

The effects of the titrant volume error on the estimates of K and n can be statistically quite significant if small titrant volumes are employed in the experiment. However, such errors are not a very likely source of the reported discrepancies between calorimetric and van't Hoff estimates of $\Delta H°$ If a similar titration procedure is used over a range of T, the result should be a roughly constant *relative* error in $K(T)$, and a constant relative error leads to no change in the van't Hoff estimates of $\Delta H°$.

In summary, the observed heat disparities in the first injections in ITC experiments can be due mainly to volume shortfall from backlash in the drive screw mechanism of the syringe. Our observations indicate that such errors can be 1 μL or greater. Analysis of ITC data under the prevailing assumption that the effects are due to preinjection mixing can lead to significant overestimation of K and n, with the effect being most severe when small injection volumes are used in the experiments. The problem can be avoided by simply preceding each run with a command for a short down motion of

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[3] Between the time of recording the early and the more recent data, the plunger height was adjusted. The increase in $-\Delta v$ from ∼1 to 1.6 μL is tentatively attributed to this change.

[4] The value of χ^2 for the least-squares fit is totally insensitive to the inclusion of the Δv parameter, since q for the first injection is always fitted exactly by the model, while it is simply neglected in the standard analysis procedure.

the plunger to absorb the backlash from the up motion of the purge/refill command.

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