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Calibration in isothermal titration calorimetry: Heat and cell volume from heat of dilution of NaCl(*aq*)

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Abstract

An isothermal titration calorimeter of the perfusion type (MicroCal model VP-ITC) is calibrated using the heat of dilution of NaCl in water. The relative apparent molar enthalpy function (L_ϕ) for NaCl(*aq*) varies strongly and nonlinearly with concentration in the low-concentration region (<0.2 M) that is sampled easily and extensively in a single program of injections of NaCl solution into water. This nonlinearity makes it possible to calibrate with respect to two quantities: the measured heat and the active cell volume. The heat factor is determined with typical standard error 0.003; its value in the current case is 0.987. The cell volume factor is 0.93 but is quite sensitive to possible systematic errors in the temperature and in the literature values for L_ϕ. Both correction factors are closely tied to the delivered volume from the injection syringe, which required a correction factor of 0.973, attributed to an instrumental gear ratio error. Temperature calibration of the instrument showed a small offset of 0.12 K at the temperature 25 °C of the experiments, but the error increased to more than 1 K at 46 °C. The experiments were not able to distinguish clearly between mixing algorithms that assume instantaneous mixing on injection and those that assume instantaneous injection followed by mixing; however, examination of these algorithms has revealed an error in a program widely used to analyze isothermal titration calorimetry data.

Keywords: ITC; Calibration; Dilution enthalpy; NaCl(*aq*); Data analysis

During recent years, the method of isothermal titration calorimetry (ITC)[1] has surged in popularity for the study of chemical binding phenomena in widely ranging applications, especially biochemical processes. In ITC, one reactant (titrant) is injected into a cell containing the other reactant (titrate) in sequential fashion, with the heat being measured for each of the typically 10–30 injections. Each injection increases the stoichiometry ratio of titrant to titrate in the cell; thus, the heat constitutes a titration curve, the analysis of which yields the enthalpy change $\Delta H°$ and the equilibrium binding constant $K°$ for the reaction. With the instrumentation available today [1–3], it is possible to estimate both of these quantities with relative standard errors less than 1% [4]. However, to realize accuracy commensurate with this precision, it is necessary to devise suitable calibration procedures that replicate the operations carried out in the binding experiments [5–7]. This is particularly true of the common perfusion instruments in which the reaction vessel has an active region and an overflow region, with material being expelled from the active region with each injection of titrant. This leads to ambiguities about the magnitude of the active volume [8], the nature of the mixing and heat generation that occur therein, and the efficiency with which that heat is measured.

In the current work, I have used heat of dilution of NaCl(*aq*) to calibrate a MicroCal model VP-ITC instrument. An important outcome of this study is the realization that this process can be used to calibrate with respect to two parameters: the heat calibration constant and either the cell or the syringe volume constant. This fortunate circumstance results from the strongly nonlinear dependence of the relative apparent molar enthalpy function L_ϕ for NaCl(*aq*) on concentration over a range of the latter that

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[1] *Abbreviations used*: ITC, isothermal titration calorimetry; LS, least squares.

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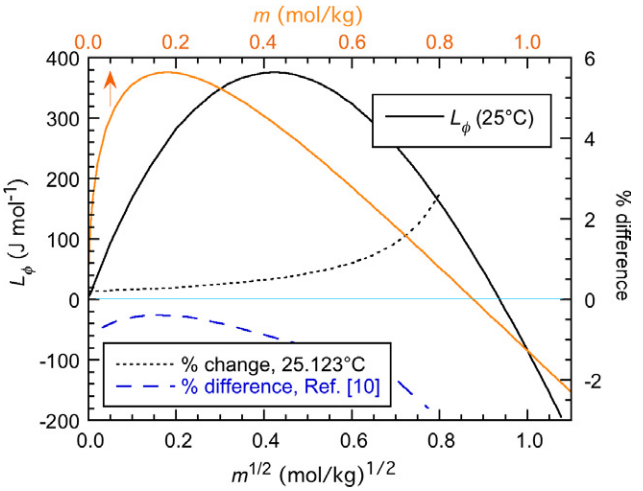

Fig. 1. Relative apparent molar enthalpy (L_ϕ) of NaCl(*aq*) at 25 °C, displayed as a function of the square root of the molality [9] and of the molality (light curve, scale at top). For comparison, the figure shows the percentage changes in this function that result from increasing T by 0.123 °C or from using the results of an earlier analysis of the NaCl(*aq*) data [10]. These results are included because the analysis is sensitive to changes this small.

is easily sampled in a single series of dilution injections (Fig. 1). Here I have calibrated the syringe volume separately and have used the dilution experiments to calibrate the heat and the active cell volume.[2]

In the course of this work, I have also examined the algorithms used to treat mixing and heat generation in the active volume V_0 of the reaction vessel. The limiting versions of these algorithms assume either (i) instantaneous injection followed by slow mixing in the cell or (ii) instantaneous mixing during each injection of titrant. In the former case (i), the material expelled from the active volume has the prior equilibrium composition; in the latter case (ii), the expelled material must include some of the titrant from the current injection. In a typical experiment, 5–30 μl of titrant is injected in 5–60 s, so the true situation should generally lie between these extremes. In an attempt to sample the dependence on this mixing assumption, I have designed experiments to include injections of widely varying volume (10–100 μl) within each run. The resulting volume calibration constant varies slightly (~1%) between the two limiting assumptions, but unfortunately, the fitting is not sufficiently sensitive to permit a statistical determination of the "happy medium". On the other hand, these efforts have revealed an error in the standard algorithm widely used to analyze ITC data. They have also confirmed the earlier indication [8] that the stated cell volume for the VP-ITC is too large; in fact, they have indicated that the error is even greater than the previously estimated 3%.

[2] If the dependence of the solution enthalpy on concentration is linear, one can show that the heat is independent of the cell volume, so that only the heat calibration constant can be obtained.

Materials and methods

Mixing algorithms and heat of dilution

In the simplest type of titration calorimeter, all injected material remains in the active region of the cell and all heat produced therein is measured. This model can be realized in practice by underfilling the cell in a perfusion instrument such as the VP-ITC. In such fixed cell instruments, one drawback of this approach is the difficulty of precisely metering the titrate into the cell at the outset, and another is possible artifacts related to the presence of two phases in the cell. Measurements conducted in this way typically are also much noisier than those with the overfilled cell arrangement, leading to the preference for the latter.

In the perfusion arrangement, the working assumption is that the material expelled from the active region V_0 never again is involved in either the mixing or the production of detected heat. Clearly, this cannot be true on the very long time scale. On the other hand, there is good experimental evidence to support its validity on the approximately 2-h time scale of a typical ITC experiment [8]. Still, because it cannot be exactly true, it does contribute to the ambiguity of V_0. Is it the same for small and large injections? For slow and fast? For any stirring speed? The current work does support the definition of a unique V_0 for small and large injections, but it offers little guidance on the role of injection rate and none on the role of stirring speed because most experiments were done at fixed injection and stirring rates.

As a lead-in to the more complex perfusion mixing models, I start by considering what happens in a simpler cumulative volume arrangement. For the very dilute solutions typically employed in ITC experiments, it is safe to assume that volumes are additive. Then, with each injection of titrant, the total volume increases in accord with

$$V_i = V_b + v_{\mathrm{cum},i} = V_b + \sum_{j=1}^{i} v_j, \tag{1}$$

where V_b is the starting volume of solution in the cell and $v_{\mathrm{cum},i}$ is the cumulative titrant volume after the ith injection, with volume v_j possibly varying with each injection. If the starting concentration of solute in the cell is $[\mathrm{X}]_0$, the concentration after i injections of titrant having concentration $[\mathrm{X}]_\mathrm{S}$ is

$$[\mathrm{X}]_i = \frac{1}{V_i}([\mathrm{X}]_0 V_b + [\mathrm{X}]_\mathrm{S} v_{\mathrm{cum},i}). \tag{2}$$

The enthalpy in a specified volume of the solution is the product of the relative apparent molar enthalpy L_ϕ (Fig. 1) at the stated concentration, and the amount of solute, or $L_\phi([\mathrm{X}]_i)\ V_i[\mathrm{X}]_i$ after the ith injection. Thus, the heat produced by the ith injection is the before/after difference,

$$q_i = L_\phi([\mathrm{X}]_i)[\mathrm{X}]_i V_i - L_\phi([\mathrm{X}]_{i-1})[\mathrm{X}]_{i-1} V_{i-1} - L_\phi([\mathrm{X}]_\mathrm{S})[\mathrm{X}]_\mathrm{S} v_i, \tag{3}$$

where the last term is recognized as the enthalpy of the solution from the syringe in the ith injection, to be designated $H_{S,i}$. Because L_ϕ is presumed to be well known, the comparison of measured and computed values of q_i constitutes a calibration for the instrument. A series of such measurements can be least squares (LS) fitted to obtain estimates of correction factors for the heat measurement and, because of the sensitivity of L_ϕ to concentration, at least one other quantity (e.g., the starting volume V_b, the concentration $[X]_S$, the titrant delivery volume v_j).

We now consider perfusion model (1). Prior to the ith injection of titrant, the concentration in the cell is $[X]_{i-1}$. When volume v_i of titrant is injected into the cell from the syringe, this same volume is expelled into the overflow region at the existing concentration $[X]_{i-1}$. With subsequent mixing, the concentration becomes

$$[X]_i = [X]_{i-1}(1 - f_i) + [X]_S f_i, \tag{4}$$

where $f_i = v_i/V_0$. The heat is again the before/after difference, but it is restricted to just the material in the volume V_0 after the injection, giving

$$q_{i,(1)} = L_\phi([X]_i)[X]_i V_0 - L_\phi([X]_{i-1})[X]_{i-1}(V_0 - v_i) - H_{S,i}. \tag{5}$$

As before, the heat may be either positive or negative, depending primarily on the relationship between the values of L_ϕ at the concentrations of the titrant and the values of the resulting solution (Fig. 1).

In model (2), the concentration in the cell is adjusting instantaneously with the injection of titrant, and it is this varying composition that is being expelled from the active volume. To treat this situation properly, we require a differential model. The injection of volume dv of titrant at concentration $[X]_S$ into the solution at $[X]$ produces a change in the concentration,

$$d[X] = \frac{dv}{V_0}([X]_S - [X]). \tag{6}$$

This equation is easily separated and integrated, yielding, for the ith injection,

$$[X]_i = [X]_S - ([X]_S - [X]_{i-1}) \exp\left(-\frac{v_i}{V_0}\right). \tag{7}$$

By examining the results of Eq. (7) for $i = 1, 2, 3, \ldots$, we find

$$[X]_i = [X]_S\left[1 - \exp\left(-\frac{v_{\mathrm{cum},i}}{V_0}\right)\right], \tag{8}$$

where $v_{\mathrm{cum},i}$ is, as before, the cumulative titrant volume.

The incremental heat associated with an injection is

$$dq = L_\phi([X] + d[X])([X] + d[X])V_0 - L_\phi([X])[X] \times (V_0 - dv) - L_\phi([X]_S)[X]_S dv. \tag{9}$$

Dropping all products containing two differentials, we obtain

$$dq = \left\{L_\phi([X]) + [X]\frac{dL_\phi}{d[X]}\right\}V_0 d[X] + \{L_\phi([X])[X] - L_\phi([X]_S)[X]_S\}dv. \tag{10}$$

Integrating $[X]_{i-1} \to [X]_i$ and $v = 0 \to v_i$, using Eq. (6) to relate dv and $d[X]$, we obtain

$$q_{i,(2)} = V_0\{L_\phi([X]_i)[X]_i - L_\phi([X]_{i-1})[X]_{i-1}\} + \int_0^{v_i} [X]L_\phi([X])dv - H_{S,i}. \tag{11}$$

If we approximate the integral as the product of v_i and the average of the integrand over the concentration range, we find

$$q_{i,(2)} = V_0\{L_\phi([X]_i)[X]_i(1 + r) - L_\phi([X]_{i-1})[X]_{i-1}(1 - r)\} - H_{S,i}, \tag{12}$$

where $r = v_i/2V_0$.

Equation (12) is consistent with the algorithm used in the Origin program supplied by MicroCal with its VP-ITC instrument. However, the equation used to evaluate concentrations in this program is not the same as Eq. (8). Rather, it is

$$[X]_i = [X]_S\frac{v_{\mathrm{cum},i}}{V_0}\left(1 - \frac{v_{\mathrm{cum},i}}{2V_0}\right), \tag{13}$$

which corresponds to taking just the first three terms in the expansion of the exponential in Eq. (8).[3] As we will see below, this leads to an inconsistency in the computation of the heat that is statistically significant in the analysis of the calibration data.

Computational methods

The nonlinear LS calculations were done using standard methods that have been described previously [4,11]; similar programs are readily available [12,13]. Data were weighted in accord with the recently determined variance function for the VP-ITC instrument [14]; however, heat production was small enough for most injections to make the weights roughly constant, so that nonconstant weighting of the data was not a very important aspect of the analysis.

From a computational standpoint, one can bridge between the two perfusion models by using Eqs. (4) and (5) from the "integral" model (1) but breaking up the actual injection volumes into smaller increments. In the current case (10 injections, $v_i = 10$–92 μl), increments of 1 μl brought the computed concentrations to within 0.04% of the Eq. (8) values, and increments of 0.01 μl gave five-figure agreement. These computations also confirmed the high reliability of the approximation of Eq. (12).

The L_ϕ function (Fig. 1) is, of course, key to any analysis of dilution data. At low molality m, L_ϕ is a natural function of $m^{1/2}$ [9,10]. However, because ITC is a volume-

[3] Equation (13) is the same as Eq. (4) in the appendix of the instruction manual for the MicroCal model VP-ITC.

based method, it is necessary to express L_ϕ as a function of concentration rather than molality. For use in the LS fitting, the literature-based L_ϕ values were fitted to polynomials in $M^{1/2}$ over the relevant concentration ranges, using solution density data from Pitzer and coworkers [15] to relate molarity to molality.

Experiments

The NaCl dilution experiments were carried out on several occasions, using concentrations of solute from 0.207 to 0.940 M. The NaCl was certified A.C.S. grade and was dried in an oven prior to weighing; later checks showed no mass change after standing in air for hours (as expected because NaCl is not strongly hygroscopic). In most of the experiments, the cell contained water at the outset; however, in some of the experiments, water was injected into a salt solution. All measurements were done using a stirring speed of 300/min at a stated temperature of 25 °C; subsequent calibration with a thermistor indicated that the true temperature was higher by 0.123 °C (see below).

The syringe calibration was checked by weighing water expelled in programmed "down" commands, preceded by a short down motion to eliminate backlash [16]. Checks showed that evaporation of water from the receptacle cells was not a significant problem in these measurements. However, the tendency of the metal syringe/stirring paddle to remain wet after such expulsions required special effort to ensure that all expelled water was accounted for properly.

Results and discussion

Mixing algorithm differences

Fig. 2 illustrates the concentration differences resulting from the different perfusion models for a typical run that uses a full syringe of titrant. Fig. 3 shows the corresponding heats as computed for the stated cell volume and starting [NaCl] with no corrections. Note that the erroneous Eq. (13) generates concentrations lower than those obtained for instantaneous mixing, although this is physically impossible. This results in heats that are significantly too low for the last few injections. The heat differences between models (1) and (2) are significant as well. However, here the heats are consistent with the mixing assumptions, whereas those based on Eq. (13) are not, as becomes clear when the different models are used to fit the experimental data (see below).

The actual thermogram for this experiment is rich in unexpected structure, as shown in Fig. 4. The heat effect initially is exothermic but then changes over to endothermic. The peaks in the switchover region show a "ringing" behavior (Fig. 4, inset), and the expanded display shows a slow return to baseline, especially for the last few large injections. Recognition of this effect led to the use of long times (~15 min) between injections, and these possibly should have been even longer for the next to last injection.

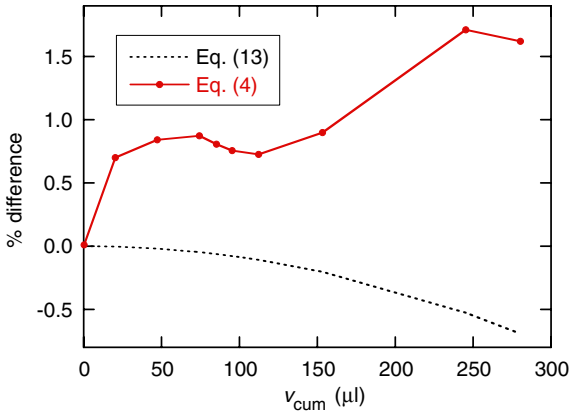

Fig. 2. Percentage differences in computed concentrations for mixing model (1) and for Eq. (13), as compared with the values obtained for instantaneous mixing model (2). The points on the upper curve indicate the actual injections. The cell volume V_0 was 1.4106 ml.

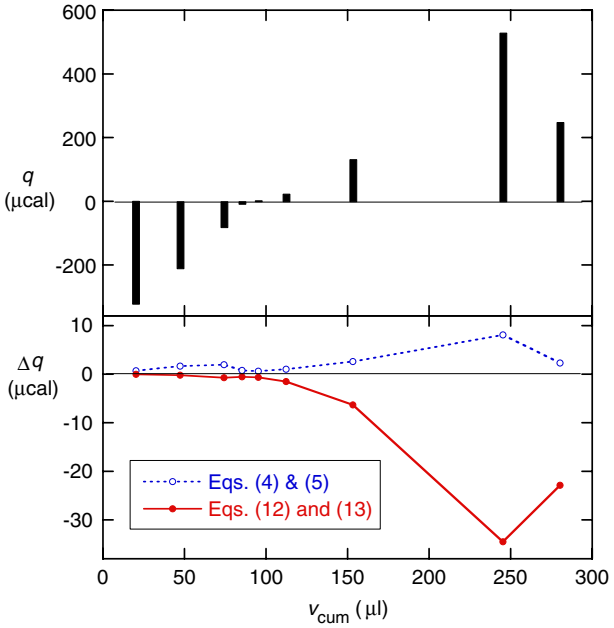

Fig. 3. Calculated q values for the same injection program, using mixing model (2) and starting with 0.376 M NaCl in the syringe and water in the cell at 25 °C. The differences obtained using the other mixing assumptions are shown at the bottom.

All of this structure is "real" in that it was replicated in other runs.

Temperature and syringe volume calibration

A calibrated (0.01 K) thermistor was used to check the fiducial cell temperature at a number of settings between 21 and 46 °C.[4] The resulting calibration curve (Fig. 5)

[4] Most temperature measurements were recorded only after the VP-ITC had gone into "thermostatting" mode, with the DT parameter essentially zero. The thermistor was gently agitated vertically to achieve some mixing and to guard against local heating by the thermistor itself. Temperatures recorded with and without such agitation agreed within 0.04 K.

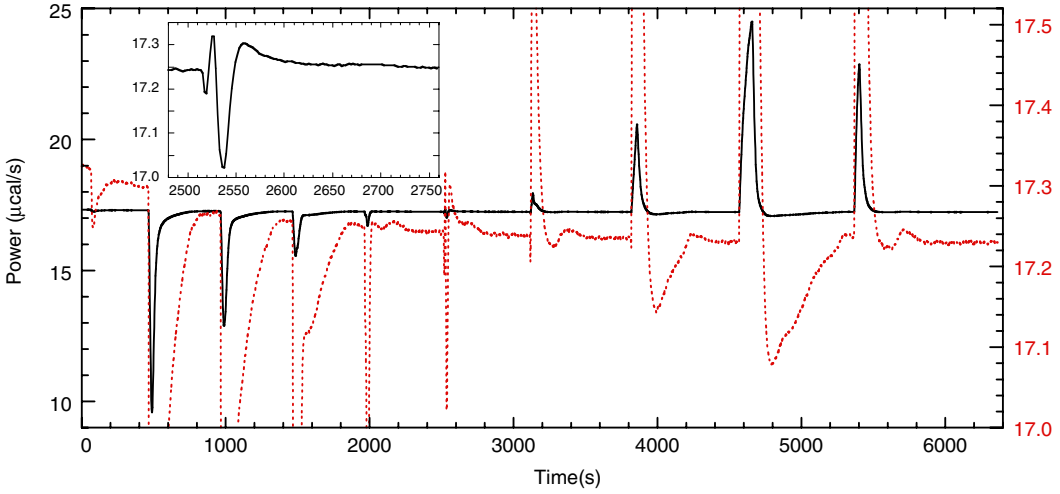

Fig. 4. Thermogram obtained for injection program illustrated in Figs. 2 and 3, with expanded scale for baseline region (scale to right) and for injection 6 (inset). The actual titrant volumes (in μl) were 0.3, 20, 27, 27, 11, 10, 17, 41, 92, and 35.

shows that the temperature is well controlled but not accurately reported by the instrument. Although the error at 25 °C is small, it is still important to the analysis, because the L_ϕ curves are very sensitive functions of T (see below).

The estimates of the syringe volume correction factor are shown in Fig. 6 for results obtained for two syringe needles on different days using different measurement procedures. The differences for the two needles are not significant practically, so the value 0.973 is adopted for most of the computations discussed below. This value is consistent with a 37/38 gear ratio error discovered by the manufacturer for some of the syringes provided with recently sold instruments.[5] Thus, the procedure behind the second set of four measurements on the first day is considered to be flawed, but its high value (0.982) is an indication of the possible systematic errors in such measurements.

The measurements behind Fig. 6 involved full or nearly full syringes in each case. At the weighing precision of a standard analytical balance (0.3 mg), this technique is not sensitive enough to check the small titrant volumes typical of individual injections. However, there is no reason to believe that the observed correction factor is anything other than the gear ratio error. Indeed, the small magnitudes of the fit residuals from the analysis (see below) confirm that the syringe volumes are delivered with high precision.

Heat and cell volume calibration

In principle, the heats estimated by integrating the peaks in thermograms such as that in Fig. 4, after correction for small water–water blanks, can be fitted to a model containing two parameters: the correction factors for the heat estimation (from the instrument's compensation power) and for the cell volume V_0. Preliminary computations indicated that these typically are determined in such fits with statistical errors of approximately 0.002. However, the analysis of data from roughly a dozen different runs showed much greater run-to-run variability and suggested that systematic errors are much more important to the overall analysis than are purely statistical errors. Systematic errors include possible solution concentration errors as well as limitations in the reliability of the L_ϕ data, the titrant volume, the temperature calibration, and the mixing model. Accordingly, I first consider just the data extracted from the thermogram in Fig. 4 and use them to illustrate the magnitude of these systematic effects.

As an aid to checking systematic effects, I have devised a four-parameter fit model that includes correction factors for the concentration of NaCl in the syringe and for the

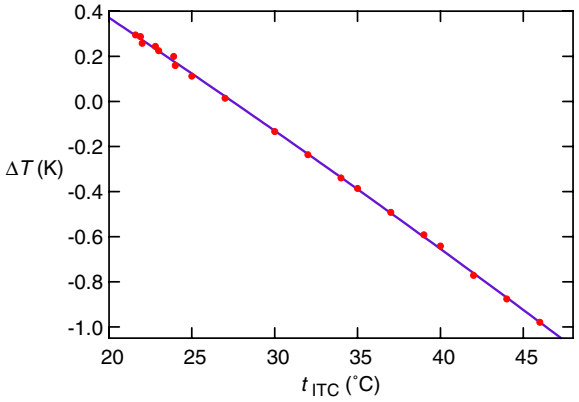

Fig. 5. VP-ITC temperature correction (true–fiducial) over the stated range. The curve is a fitted quadratic in the argument (t – 25 °C) and gives an error of 0.123(3) K at 25 °C. Most values beyond 25 °C were recorded after the VP-ITC instrument had equilibrated at the specified temperature.

[5] I became aware of the possibility of this problem through private communications with representatives of MicroCal that largely inspired these measurements. The error was confirmed by Mike Brandts of MicroCal and can be fixed in the instrument by altering the "steps per inch" parameter under the "constants" tab in the VPViewer program.

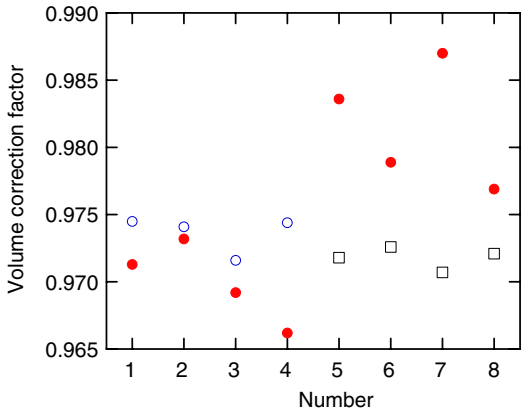

Fig. 6. Syringe volume correction factor, as obtained by weighing water expelled in programmed down motions of 2.2–2.6 in. Solid points were obtained on one day using two different collection and measurement strategies, whereas open points were obtained on another day using a third approach and (for squares) a second syringe needle. The average factors for the open points were 0.9736(7) (circles) and 0.9718(4) (squares).

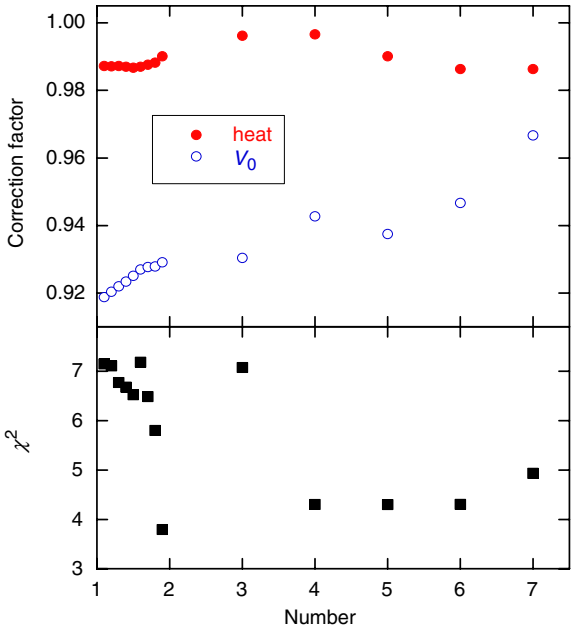

Fig. 7. Results from analysis of thermogram of Fig. 4 for various treatments and assumptions. Points between numbers 1 and 2 show the dependence on mixing algorithm, from model (2) to model (1), obtained by using Eqs. (4) and (5) but breaking each injection into fixed Δv pieces and then increasing the increment from 1 to 100 μl. All other values employed $\Delta v = 10$ μl, with one condition changed: (3) titrant volume correction factor increased to 0.982; (4) [NaCl] correction factor fitted, value = 1.006(3); (5) H_S correction factor fitted, value = 0.996(2); (6) L_ϕ at 25 °C used in analysis; (7) L_ϕ from Ref. [10] at 25.123 °C used.

enthalpy H_S of the concentrated solution in the syringe (in most experiments).[6] The reason for the latter is that it is the easiest way to correct for limitations in the L_ϕ function because the differences from different determinations and for different temperatures tend to increase with increasing concentrations (Fig. 1). It is interesting that one can actually fit a correction factor for the solution concentration (i.e., treat the solution concentration as unknown). The reason for this is the sensitive dependence of L_ϕ on the range of concentrations of the titrant and the solutions that result from dilution. However, because this effect depends strongly on L_ϕ of the titrant (and hence H_S), it is not possible to simultaneously fit both of these correction factors with any reliability.

Results from such calculations are illustrated in Fig. 7. The V_0 correction factor is much more sensitive to systematic effects than is the heat calibration. The V_0 factor varies by approximately 1% with changes in the mixing model, but it increases more significantly with the other changes, particularly the choice of L_ϕ function. The heat calibration spans a more limited range of approximately 1%; both the heat calibration and the volume factor track changes in the titrant volume almost exactly, with other factors being held constant. The χ^2 values are reasonable for this data set (nine fitted values). The observation of lowest χ^2 for the purely integral mixing model (1) (point nearest number 2 in Fig. 7) was somewhat surprising, since the longest injections surely involved times long enough to permit expulsion of some new material. Yet this result was observed for a number of other data sets as well.

The results obtained for the mixing approximation of Eq. (13) are not included in this display because they are off scale: 1.06 and 0.89 for heat and volume calibration factors, respectively, and 274 for χ^2. Similar behavior occurred when this equation was used on other data sets that included large injections and was the basis of the earlier comments about the deficiency of this approximation. The simple cumulative mixing model of Eqs. (1)–(3) likewise was shown to be unsuitable, although surprisingly the χ^2 increase was smaller—a factor of only ~5. Still, the resulting correction factors for the volume were unreasonable: 0.88 as compared with expected values of approximately 1.3 for the known cell and overflow volumes of 1.4 and 0.4 ml, respectively.

Fig. 8 illustrates the range of correction factors obtained from different dilution runs, with the data processed as for the middle of the first group of points in Fig. 7. The heat factors center around 0.987 except for the last two groups of experiments. Of the latter, the 5th group of values were obtained using an injection program like that used to obtain Figs. 4 and 7 but with shorter time intervals between injections. As a result, the last two peaks in each run were far from baseline prior to the next injection, and so they were deleted from the analyses. It is possible that the ~1% smaller heats for the remaining points resulted from a similar, but less obvious, incomplete return to baseline. The last group of values came from experiments conducted

[6] The adjustable parameters (C_1, C_2, …) are accommodated in the nonlinear LS fits by defining the measured heat as the nominal value × C_1, the active volume as $V_0 \times C_2$, the titrant concentration as $[X]_S \times C_3$, and the titrant enthalpy as $H_S \times C_4$.

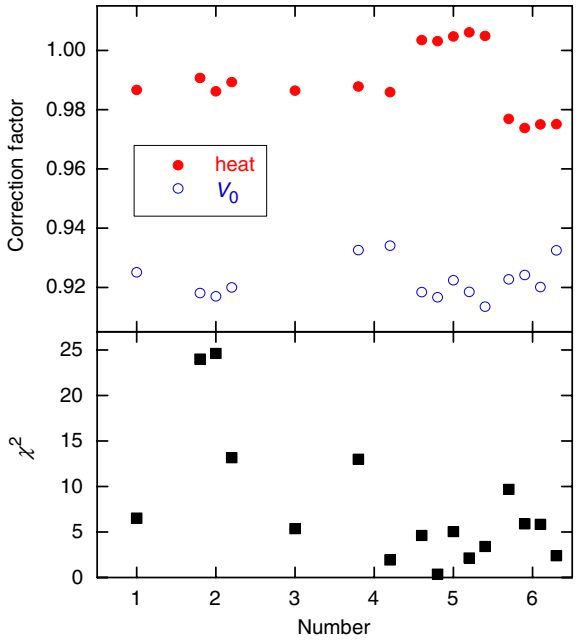

Fig. 8. Results from different dilution experiments, analyzed as for the points at $x = 1.5$ in Fig. 7, that are reproduced here as number 1. Others: (2) 0.376 M NaCl injected into water, with $v_i = 7, 13, 19, \ldots, 55$ µl; (3) water injected into 0.940 M NaCl, with $v_i = 3$–6 µl; (4) 0.2092 M NaCl injected into water using same program as used in Fig. 4; (5) 0.376 M NaCl into water, using same injection program as in Fig. 4 but shorter delay between injections; (6) same injection program but 0.2067 M NaCl into water.

6 months earlier than all others, and it is possible that the heat factor for the instrument was the indicated ~1% smaller at that time.

The V_0 factors center around 0.925, with the exception of the V_0 factor for number 3, which is off the scale of this plot at 1.04 ± 0.15. The very large uncertainty in this case is inherent in the relevant experiment, which involved injection of water into concentrated NaCl. The resulting data sample only a narrow concentration range of L_ϕ, where it is roughly linear in the concentration. The V_0 factor would be completely indeterminate if this relationship were purely linear, as discussed earlier in note 2. On the other hand, the heat factor is almost completely decoupled from V_0 and so is well determined, and it agrees closely with the results for all but the last two sets of experiments.

The results in Fig. 8 were checked for only the most important systematic effect investigated in Fig. 7, namely the dependence on source of L_ϕ. When the determination from Clarke and Glew [10] was used in place of that from Archer and Carter [9], most data sets responded in a way very similar to that shown in Fig. 7, with an increase in the V_0 correction factor by approximately 0.04 and no significant change in the heat factor.

This strong sensitivity to the L_ϕ function is both surprising and unfortunate. It is surprising because the differences in the two functions are less than 1% over much of the range of concentration involved in most experiments. It is unfortunate because, as noted earlier, the inherent statistical error in the V_0 correction factor normally is more than an order of magnitude smaller than the L_ϕ dependence. The Archer and Carter determination [9] is more recent and is based on a proper and more comprehensive analysis of the large body of existing data. On the other hand, Archer's 1992 analysis [17] involved a very similar treatment of many of the same data and yielded an L_ϕ curve that falls roughly midway between the Archer and Carter [9] and Clarke and Glew [10] curves in the $m = 0.04$–0.4 molal range relevant to most of the current experiments. Thus, these differences provide an indication of the remaining uncertainty in the L_ϕ function for NaCl(*aq*) near 25 °C.

We can compare the current V_0 correction factors with the earlier determination based on reaction stoichiometry and physical measurements [8]. That value was 0.973 but was made without knowledge of the syringe volume correction. If we apply the latter to the stoichiometry-based estimate, the value becomes 0.946, which roughly splits the estimates from the two L_ϕ functions. On the other hand, it then becomes hard to explain the volume effect physically (previously attributed to volume displacement by the syringe assembly). Perhaps there is a larger excluded volume that develops in the form of a cycling vortex driven by the rotating shaft of the stirring assembly. As mild support for such a notion, I have observed anomalous apparent heat in water–water blanks when injecting rapidly, and this could possibly relate to turbulent disruption of such a vortex. Clearly, this matter will require further study.

Conclusion

The heat of dilution of NaCl in water has been used to calibrate an isothermal titration calorimeter of the perfusion type (MicroCal VP-ITC). Because the relative apparent molar enthalpy L_ϕ of NaCl(*aq*) is strongly nonlinear in its dependence on concentration in the experimentally sampled range, it is possible to determine not only the heat calibration constant but also a volume correction factor, in this case the active cell volume. The heat calibration factor is determined with typical precision of 0.003; from a number of different experiments, its value in the current case was 0.987. The cell volume correction factor is, in principle, determined with similar precision but is strongly sensitive to possible systematic errors, especially temperature (~0.02 for 0.1-K change) and possible inadequacies in the literature assessments of L_ϕ on which the analysis relies. Use of the most recent values leads to a value of 0.93 for the cell volume correction factor, and this is roughly consistent with the estimate of 0.95 from a previous assessment [8] after correction for syringe volume error.[7]

Because of the importance of temperature and syringe delivery volume to the calibration, those quantities were

[7] Computer programs for computing the relative apparent molar enthalpy function for NaCl(*aq*) as a function of T for a pressure of 1.00 bar, and for analyzing NaCl(*aq*) dilution data from a perfusion-type microcalorimeter, are available on request.

measured separately. The syringe volume factor was 0.973 and stems from a gear ratio error of 37/38 in the instrument parameters. The temperature calibration showed an offset of 0.12 K at 25 °C, which is within the stated instrument specifications (0.2 K).[8] However, the error increases away from 25 °C and amounts to a net change of 1 K in 20 K. That represents a 5% error in any property that depends on the derivative of temperature such as heat capacity and reaction enthalpy obtained from a van't Hoff analysis of the T variation of the equilibrium constant. In particular, this effect can account for much of the remaining discrepancy between calorimetric and van't Hoff estimates of ΔH° in a recent study of the Ba^{2+}/crown ether complexation reaction [18].

The current calibration analysis is not sensitive to variations in the cell mixing algorithm between the limiting assumptions of instantaneous mixing and those of instantaneous injection of titrant. However, close examination of these models has revealed a flaw in the titrant mixing equation in the packaged software widely used to analyze ITC data. The effect of the error is negligible for small injections of titrant, but it accumulates with total titrant volume and becomes appreciable when the latter becomes approximately 100 μl. Because many experiments program total titrant volumes at least twice this size, this error should be fixed.

Experiments like those reported here for NaCl were conducted also with $BaCl_2$ and K_2SO_4 using best available literature for the respective L_ϕ functions. Analysis of the results showed very large χ^2 values and systematic effects in the residuals that persisted even when the correction parameter for H_S was included in the fit model. I conclude that the literature assessments for these solutes cannot be trusted for this analysis. The "good news" of this result is that, with confident knowledge of the active cell volume V_0, this instrumentation is capable of yielding L_ϕ assessments with unprecedented reliability. Although there is little excitement these days in measuring this property, it is important to the overall determination of the thermodynamics of aqueous salt systems [10].

From the thermodynamics standpoint, NaCl(*aq*) is the "gold standard" of aqueous salt systems, so there is nothing we can turn to with confidence to replace it as an ionic solute calibrant. It would be nice to have direct corroboratory measurements of its L_ϕ function with the kind of precision that the current ITC instrument is capable of providing. In the meantime, it appears that the cell volume can be calibrated more reliably using reaction stoichiometry in simple 1:1 binding, as in the Ba^{2+}/crown ether reaction used previously for this purpose [8].

[8] The VP-ITC temperature calibration can be checked within 0.2 K at two temperatures near 25 and 70 °C by operating the instrument in "DSC mode" and observing the melting behavior of two samples supplied by the manufacturer. Such checks (by other researchers) have shown agreement for our instrument within the stated 0.2 K at the two calibration points, suggesting that the calibration function is flawed away from these points.

The results in the thermogram of Fig. 4 show that it is necessary to wait much longer for return to baseline than is customary in most ITC work. This observation was borne out in the analysis of the data, and it bodes ill for hopes to achieve high-quality results from the "single injection method" [19]. On the other hand, the important result that in 1:1 binding precision is enhanced by fewer injections rather than more injections [4] takes on even more practical significance. Recent work has shown that properly designed experiments with just the minimum 3 injections can improve precision by another order of magnitude over results obtained with the recommended 10-injection protocol from Ref. [4,20].

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