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Significant discrepancies between van't Hoff and calorimetric enthalpies. III

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Abstract

The study of a wide variety of reversible reactions in solution indicates that the enthalpy, ΔH_{vH}, which controls the temperature variation of the equilibrium constant for a reaction, can seldom, if ever, be taken to be independent of the temperature. It is also found in most cases that the values for ΔH_{vH}, properly evaluated as varying with the temperature, differ significantly from the values for the enthalpy, ΔH_{cal}, determined by direct calorimetry under the same experimental conditions. In a continuing search for reactions which show agreement between ΔH_{vH} and ΔH_{cal}, we have studied by isothermal titration calorimetry the reactions of heptylamine with heptanoic acid in dodecane solution and of α-cyclodextrin with sodium heptanoate in aqueous solution.

Keywords: Calorimetric enthalpy; Isothermal titration calorimetry; van't Hoff enthalpy

1. Introduction

A large proportion of the enthalpy values that have appeared in the scientific literature through the years has been derived from data on the temperature variation of equilibrium constants interpreted by means of the van't Hoff equation

$$\frac{d \ln K}{d(1/T)} = \frac{\Delta H_{vH}}{R} \qquad (1)$$

where T is the absolute temperature and R is the gas constant. In the great majority of these applications it has been assumed that the so-called van't Hoff enthalpy is independent of temperature, and it has been evaluated as the slope of the plot of $\ln K$ as a function of $1/T$ divided by R.

Recent development has made available an excellent instrument for isothermal titration calorimetry (ITC) [1] with which one can obtain quite accurate values for both equilibrium constants and enthalpies. Such data permit direct comparisons of van't Hoff and calorimetric enthalpies obtained under identical experimental conditions. Recent studies [2,3] on a variety of systems have shown that the assumption of linear van't Hoff plots is frequently incorrect, even in cases where linear least squaring has yielded a correlation coefficient sufficiently close to unity to appear to validate the assumption. It is advisable that in all cases equilibrium data be analyzed in terms of the integrated van't Hoff equation containing at least a temperature-independent heat capacity change, ΔC_p

$$\ln \frac{K}{K_0} = \frac{\Delta H_0 - T_0 \Delta C_p}{R} \left(\frac{1}{T_0} - \frac{1}{T} \right) + \frac{\Delta C_p}{R} \ln \frac{T}{T_0} \qquad (2)$$

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Table 1
Titration of *n*-heptanoic acid with *n*-heptylamine in dodecane solution

Temp. (°C)	N	10^{-3} K (M^{-1}) Obsd.	Calcd.	ΔH_{vH} (kcal mol^{-1})	ΔH_{cal} (kcal mol^{-1})
5.5	0.877 ± 0.003	4.82 ± 0.10	4.85	−4.05	−17.72 ± 0.09
10.0	0.872 ± 0.004	4.44 ± 0.12	4.26	−4.85	−17.42 ± 0.12
15.0	0.869 ± 0.004	3.50 ± 0.08	3.62	−5.75	−17.68 ± 0.11
15.0	0.882 ± 0.005	3.73 ± 0.12	3.62	−5.75	−16.97 ± 0.14
15.0	0.883 ± 0.003	3.50 ± 0.06	3.62	−5.75	−17.46 ± 0.09
15.0	0.870 ± 0.004	3.56 ± 0.07	3.62	−5.75	−17.29 ± 0.11
20.0	0.884 ± 0.004	2.96 ± 0.06	3.01	−6.64	−16.81 ± 0.11
20.0	0.899 ± 0.006	3.09 ± 0.10	3.01	−6.64	−16.41 ± 0.15
21.0	0.895 ± 0.005	2.82 ± 0.07	2.90	−6.82	−16.42 ± 0.14
25.0	0.918 ± 0.007	2.39 ± 0.08	2.46	−7.53	−15.85 ± 0.19
25.0	0.915 ± 0.010	2.52 ± 0.12	2.46	−7.53	−15.69 ± 0.24
25.0	0.912 ± 0.010	2.62 ± 0.13	2.46	−7.53	−15.64 ± 0.25
25.0	0.923 ± 0.005	2.47 ± 0.06	2.46	−7.53	−15.86 ± 0.13
30.0	0.954 ± 0.009	2.00 ± 0.07	1.97	−8.43	−14.98 ± 0.21
30.0	0.937 ± 0.006	1.88 ± 0.04	1.97	−8.43	−15.17 ± 0.15
35.0	1.030 ± 0.010	1.56 ± 0.05	1.55	−9.32	−13.09 ± 0.20
35.0	1.030 ± 0.009	1.54 ± 0.04	1.55	−9.32	−13.21 ± 0.18

$\Delta H_{cal} = -17.402 - 0.0733t + 0.0054t^2$ (kcal mol^{-1}) (where t is in degrees Centigrade); the standard deviation is ±0.22 kcal mol^{-1}. Standard deviation in K is ±99 M^{-1}.

where T_0 is an arbitrary reference temperature, and K_0 and ΔH_0 are the assumed values at that temperature which then serve, along with ΔC_p, as adjustable parameters in the non-linear least squaring of the data. In cases where an unusually large temperature range is involved, it may be necessary to include the temperature variation of ΔC_p. We have not as yet found a system for which ΔH_{vH}, properly evaluated, and the directly observed calorimetric enthalpy, ΔH_{cal}, agree. In most cases, the disagreement is well beyond experimental uncertainty. In the present paper we briefly review certain features of our earlier work and include reports of ITC data on two apparently simple reactions, i.e. the interaction of *n*-heptanoic acid with *n*-heptylamine in dodecane solution, and of sodium heptanoate with α-cyclodextrin in aqueous solution with no added buffer.

2. Materials and methods

n-Heptylamine (99 + % pure), *n*-heptanoic acid (99% pure), and dodecane (99 + % pure; water content less than 0.005%) were purchased from Aldrich Chemical Co. Sodium hydroxide was of analytical grade from Mallinkrodt. Sodium heptanoate was prepared by titrating sodium hydroxide solution into heptanoic acid to pH 8.

All calorimetric titrations were performed using

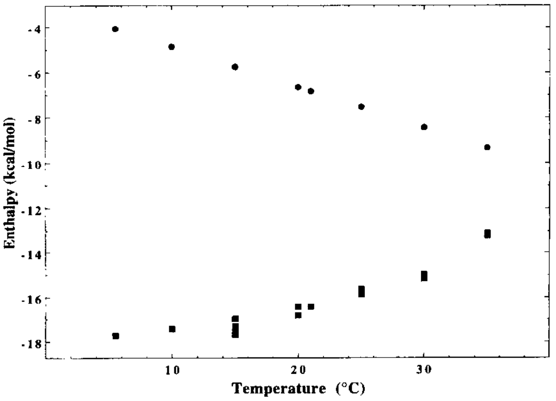

Fig. 1. The calorimetric enthalpies (■) and the van't Hoff enthalpies (●) for the reaction of *n*-heptanoic acid with *n*-heptylamine in dodecane solution plotted as functions of the temperature.

the Omega calorimeter manufactured by Microcal, Inc., Northampton, MA, and the data were evaluated by the Origin software supplied with the calorimeter.

The observed values of ln K were fitted to the integrated van't Hoff equation, as briefly noted above, using a program written in GW-BASIC.

3. Results

The binding of $BaCl_2$ by 18-crown-6 was studied in the temperature range 7–40°C in aqueous solution with no buffer present [3]. The standard deviation between the calculated and observed values for the

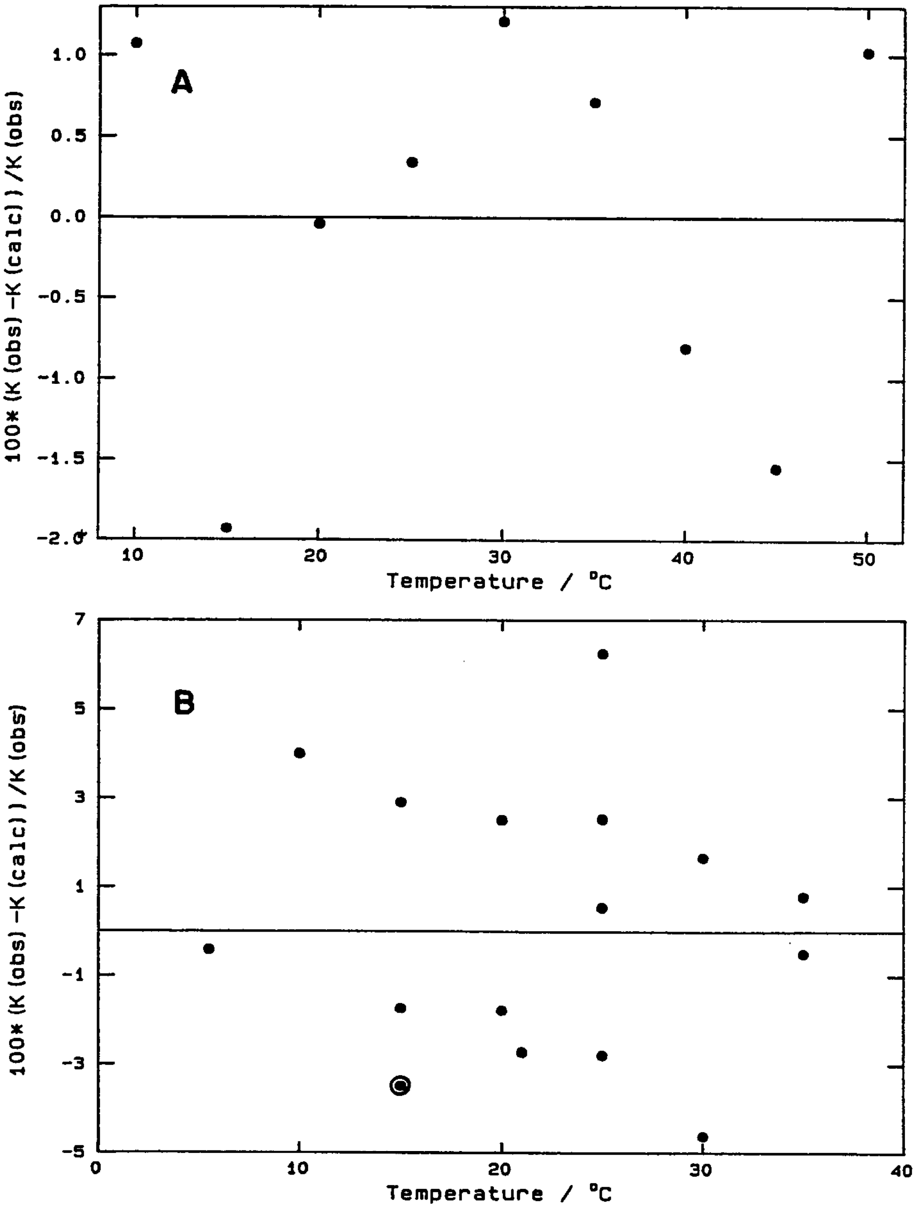

Fig. 2. Plots against temperature of the percentage fractional difference between the observed and calculated values of the binding constant. Panel A, the cyclodextrin–heptanoate system; panel B, the heptoic acid–heptylamine system.

equilibrium constant was 111 M^{-1}, which is 5% of the smallest value for the equilibrium constant. In this case, linear least squaring of ln K vs. $1/T$ gave a correlation coefficient of 0.998, but even so, ΔH_{vH} became more positive by 3.4% over the studied temperature range.

Titrations of RNase A with 2′-CMP [2] in 0.20 M potassium acetate buffer, pH 5.5, with added 0.20 M potassium chloride in the presence of 0.50 M sucrose gave values of $\Delta H_{vH}/\Delta H_{cal}$ varying from 0.68 at 15°C to 0.95 at 40°C, with ΔC_p (vH) = −287 cal K^{-1} mol^{-1} and ΔC_p (cal) = −198 cal K^{-1} mol^{-1}.

Table 1 lists the results of a calorimetric titration of heptanoic acid into heptylamine in dodecane solution, and of the van't Hoff analysis of the variation with temperature of the apparent binding constants. The calculated values of K listed in the fourth column of the table and the van't Hoff enthalpies in the fifth column were obtained by fitting the data to the integrated van't Hoff equation.

The reaction

$$CH_3(CH_2)_5COOH + H_2N(CH_2)_6CH_3 \rightleftarrows CH_3(CH_2)_5COO^-N^+H_3(CH_2)_6CH_3$$

in an anhydrous solvent was selected in the expectation that it would prove to be a simple reaction leading to agreement between ΔH_{vH} and ΔH_{cal}. Actually it turned out to be a rather complicated reaction, showing the largest differences between ΔH_{vH} and ΔH_{cal} that we have so far encountered.

The values for the calorimetrically observed enthalpies show considerably more curvature as a function of temperature than is usual. They are adequately fitted by the quadratic equation given at the bottom of the table.

The natural logarithms of the observed values for K, listed in the third column of the table, fit the usually assumed linear plot vs. $1/T$ with a correlation coefficient of 0.978, leading to an apparent value of −6.79 kcal mol^{-1} for ΔH_{vH}. As seen in the fifth column of the table, the values obtained from our non-linear treatment range in magnitude from 0.60 to 1.37 times this value. Even more striking is the fact that ΔH_{cal} becomes less negative with increasing temperature while ΔH_{vH} becomes more negative. The enthalpies are plotted as functions of the temperature in Fig. 1. The fractional differences between the observed and calculated values of the binding constant, expressed as percentages, are shown in panel B of Fig. 2. It is evident that a fairly good fit was obtained in the non-linear regression, with an average difference of 2.5%.

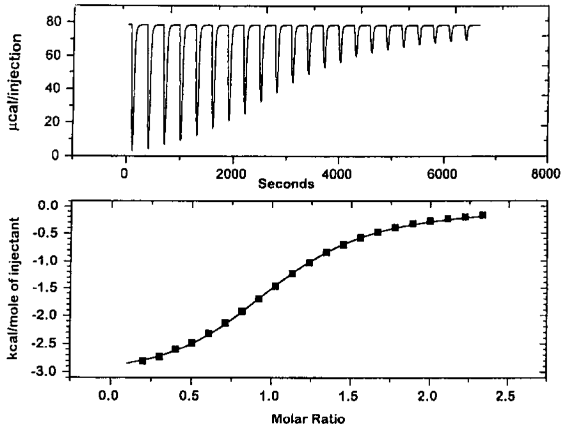

Fig. 3. Experimental data for a titration of 160.4 mM sodium n-heptanoate into 9.76 mM α-cyclodextrin in aqueous solution. Upper panel: 22 injections, each 8 µl, of n-heptanoate at 5 min intervals. Lower panel: data points obtained by the integration of injection peaks, and the titration curve obtained by fitting the data points with the Omega software. The first injection peak was discarded because of diffusion from the injection syringe during thermal equilibration before the start of the titration.

Fig. 3 shows the experimental data for a titration of 160.4 mM sodium n-heptanoate into 9.76 mM α-cyclodextrin in aqueous solution with no buffer present. Table 2 lists the data obtained in nine titra-

Table 2
Titration of α-cyclodextrin with sodium n-hepanoate in aqueous solution

Temp. (°C)	N	K (M^{-1}) Obsd.	K (M^{-1}) Calcd.	ΔH_{vH} (kcal mol^{-1})	ΔH_{cal} (kcal mol^{-1})
10	1.01 ± 0.004	968 ± 20	958	−2.29	−2.72 ± 0.01
15	1.00 ± 0.004	870 ± 18	887	−2.70	−3.20 ± 0.02
20	1.01 ± 0.003	813 ± 13	813	−3.11	−3.59 ± 0.02
25	1.01 ± 0.003	742 ± 11	739	−3.51	−3.95 ± 0.02
30	1.01 ± 0.003	675 ± 9	667	−3.92	−4.35 ± 0.02
35	1.01 ± 0.003	601 ± 8	597	−4.33	−4.73 ± 0.02
40	0.988 ± 0.003	526 ± 7	530	−4.73	−5.12 ± 0.03
45	0.983 ± 0.004	461 ± 7	468	−5.14	−5.53 ± 0.03
50	0.983 ± 0.007	415 ± 11	411	−5.55	−5.86 ± 0.06

ΔC_p (for ΔH_{vH}) = −81.4 ± 11.2 cal K^{-1} mol^{-1}.
ΔC_p (for ΔH_{cal}) = −78.0 ± 2.3 cal K^{-1} mol^{-1}.
Standard deviation in K is ± 11.6 M^{-1}.

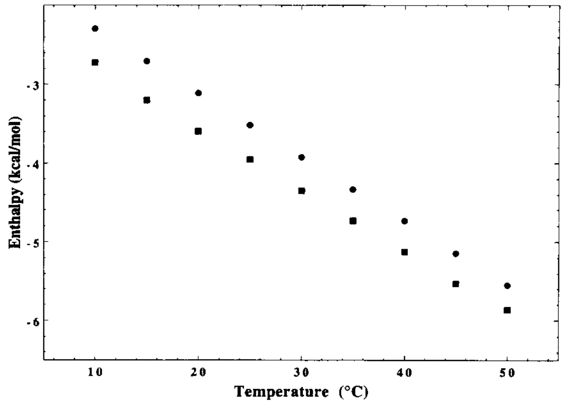

Fig. 4. The calorimetric enthalpies (■) and the van't Hoff enthalpies (●) for the reaction of α-cyclodextrin with *n*-sodium heptanoate in aqueous solution plotted as functions of the temperature.

tions of sodium *n*-heptanoate with α-cyclodextrin. Calorimetric titrations of sodium *n*-heptanoate with α-cyclodextrin at three temperatures have been previously reported by Gomez-Orellana et al. [4] with values for ΔH_{cal} agreeing closely with our values. With this system, we found as good an agreement between calorimetric and van't Hoff data as we have so far observed. The observed and calculated binding constants agree with a standard deviation of ± 8.4 M^{-1} and the van't Hoff and calorimetric enthalpies, shown in Fig. 4, agree with a standard deviation of ± 0.45 kcal mol^{-1}. In this case, the correlation coefficient of the linear $\ln K_{\mathrm{B}}$ vs. $1/T$ plot was about the same as in the heptanoic acid–heptylamine case, although here, the van't Hoff and calorimetric ΔC_{p} values differ by only 4.3%. We are unable to explain the nearly "normal" behavior of this system. It is seen in Fig. 2 that in this case, a good fit was obtained by non-linear least squaring, with an average difference of only 1.0%.

4. Discussion

It has been pointed out by Weber [5,6] that the usual form of the van't Hoff equation is, strictly speaking, incorrect in that it overlooks the contribution to the total entropy of the system, $S = S_i + S_{\mathrm{H}}$, of the entropy, S_{H}, of the surroundings of the chemical system of reacting species. This source of error is probably negligible in all chemical processes taking place at constant temperature and constant pressure, at least at pressures not far from atmospheric pressure.

It seems unlikely that deviations of reactants from unit activity coefficients can be a significant cause of discrepancies between ΔH_{vH} and ΔH_{cal}. For example, in the binding of $\mathrm{BaCl_2}$ to 18-crown-6 [3] discussed above, the inclusion of activity coefficients for $\mathrm{BaCl_2}$ at 0.009 M, the concentration at the equivalence point in the titration, ranging from 0.739 at 7°C to 0.728 at 40°C [7], made the values for ΔH_{vH} uniformly more negative by only 1%.

In the case of complex systems such as the binding of a ligand, L, to a protein, P, in a buffered aqueous solution, several processes not explicitly indicated by the simple equation $\mathrm{P} + \mathrm{L} \rightleftarrows \mathrm{PL}$, might make significant contributions to the observed reaction [8]. Suppose for example, that buffer component A is to some extent taken up by PL, buffer component B is released from P on complex formation, and that water molecules are released on complex formation. The overall process is then

$$\Delta\alpha\mathrm{A} + \mathrm{P} + \mathrm{L} \rightleftarrows \mathrm{PL}(+\Delta\alpha\mathrm{A} - \Delta w\mathrm{H_2O} - \Delta\beta\mathrm{B}) + \Delta\beta\mathrm{B} + \Delta w\mathrm{H_2O}$$

and the equilibrium constant is

$$K_T = \left(\frac{[\mathrm{PL}]}{[\mathrm{P}][\mathrm{L}]}\right)\left(\frac{\gamma_\beta^{\Delta\beta}[\mathrm{B}]^{\Delta\beta} a_{\mathrm{H_2O}}^{\Delta w}}{\gamma_{\mathrm{A}}^{\Delta\alpha}[\mathrm{A}]^{\Delta\alpha}}\right) = K_{\mathrm{app}} K'$$

where γ_{A} and γ_{B} are the activity coefficients of A and B and $a_{\mathrm{H_2O}}$ is the activity of water. The van't Hoff plot only includes $\ln K_{\mathrm{app}}$; the term $\ln K'$ may well make a significant contribution. The term ΔH_{cal} of course includes all enthalpic contributions from the undetected reactions.

Similarly, there may well be significant changes in the extents of hydration of both $\mathrm{BaCl_2}$ and 18-crown-6 when these species interact, or of sodium heptanoate and α-cyclodextrin in that interaction. It would obviously be very difficult to pursue these possibilities in any quantitative manner. The case of the reaction of heptanoic acid with heptylamine in dodecane is even more puzzling.

There can be no question, of course, concerning the fundamental correctness of the full van't Hoff equation under ordinary circumstances. The discrepancies outlined here, and the numerous others we have encountered, seem to leave us with the discouraging conclusion that chemical reactions, at least in solution, are quite generally more complex than indicated by the simple chemical equations. It seems we have no basis for deciding which enthalpy value, ΔH_{vH} or ΔH_{cal}, is the better value in any particular case.

References

[1] T. Wiseman, S. Williston, J. Brandts and L. Liu, Anal. Biochem., 179 (1989) 131–137.
[2] H. Naghibi, A. Tamura and J.M. Sturtevant, Proc. Natl. Acad. Sci. USA, 92 (1995) 5597–5599.
[3] Y. Liu and J.M. Sturtevant, Prot. Sci., 4 (1995) 2559–2561.
[4] I. Gomez-Orellana, D. Hallén and M. Stödeman, Calorimetry Conf. Abstr., 268 (1995).
[5] G. Weber, J. Phys. Chem., 99 (1995) 1052–1059.
[6] G. Weber, Proc. Natl. Acad. Sci. USA, 93 (1996) 7452–7454.
[7] S. Ardizzone, P. Longhi, T. Mussini and S. Rondinini, J. Chem. Thermodyn., 8 (1976) 557–564.
[8] D.M. Crothers, personal communication, 1996.