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Titration Calorimetry

From Concept to Application

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Preface

This brief provides an introduction to calorimetry in general and to titration calorimetry specifically. The intended audience is college students and others who are unfamiliar with calorimetry, but have some chemistry or physics background. Many graphics are included to provide a visual representation of the data that can be obtained from titration calorimetry. As a brief presentation, this document is deliberately short on details, since that would make it too long for easy access to the basic information. However, key references and a bibliography are provided for those who wish to learn about the subject in more depth. A self-test is also provided to further illustrate the principles of titration calorimetry and analysis of the data produced.

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Contents

Chapter 1
Introduction to Calorimetry

Calorimetry literally means "heat measurement." For consistency with other forms of energy and to avoid confusion, the modern unit for heat is the joule which equals 0.2390 calories. The unit for heat rate is the watt or J/s with prefixes m for milli (10^{-3}), μ for micro (10^{-6}), and n for nano (10^{-12}). Heat can be measured in only three ways, referred to as "temperature change," "heat conduction," and "power compensation" (Hansen 2001). Table 1.1 lists characteristics of these methods.

The raw data from a temperature change calorimeter is in the form of temperature versus time, and the raw data from heat conduction and power compensation calorimeters is in the form of heat rate versus time; see Fig. 1.1. This difference in the way data are produced by different types of calorimeters has led to two different ways of displaying data after being processed, i.e., as a plot of total heat, Q, or of heat per increment of titrant, dQ/dn, versus the mole ratio of titrant to titrate as shown in Fig. 1.2. Understanding the relation of these plots to the stoichiometric (n) and thermodynamic parameters (K and $\Delta_r H$) obtainable from calorimetric titration data is key to understanding the practice and applications of titration calorimetry. Therefore, wherever appropriate, the figures in this brief are presented as species distribution, total heat (Q), and heat per increment of titrant (dQ/dn) and as functions of the solution composition. The raw data from calorimetric titrations are often displayed as temperature change or heat rate versus time to illustrate the time course of the reaction. Such plots are useful for providing a sense of the time constants of the calorimeter and reaction and assessing data quality since noise in the calorimetric signal is readily apparent. However, this information is instrument and experiment specific and outside the scope of this brief. For determinations of thermodynamic quantities, the raw data plots must be converted into plots of total heat or heat per injection, and plotting these data versus time or data point number should be discouraged because the direct relations between the data and stoichiometry and thermodynamic parameters are hidden in such plots. Also, note that there are two sign conventions for heat; the American sign convention defines heat as positive for an exothermic reaction, and the European convention defines heat as negative for an

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Table 1.1 Characteristics of the three methods for calorimetric measurement of heat. Note that the "response time" is approximately six times the "time constant"

Method	Detection limit	Response time	Governing equation	Notes
Temperature change	50–500 μJ/mL	0.5–1 s	$Q = (\Delta T)$(heat capacity of vessel + contents)	ΔT must be corrected for heat exchange with the surroundings. Detection limit increases with volume
Heat conduction	0.1–50 μJ/s or μW	5–50 min	$Q = c\Delta T$	The calorimetric constant, c, is affected by heat exchange with surroundings that does not go through the ΔT sensor
Power compensation	1–10 nJ/s or nW	10–500 s	$Q = c\Delta$Power	Heat rate is calculated from changes in the input power to a heater/cooler used to maintain isothermal conditions. The calorimetric constant, c, must still be determined chemically because of possible heat exchange with surroundings

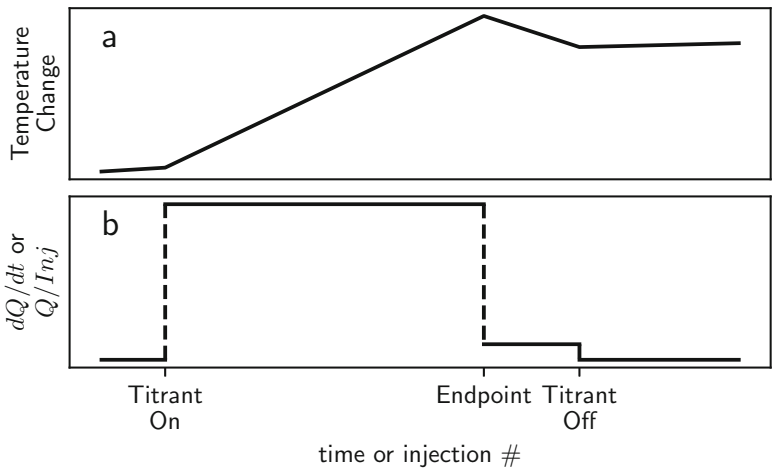

Fig. 1.1 Schematic plots of the raw data from (**a**) temperature change and (**b**) heat conduction and power compensation calorimeters for a quantitative chemical reaction. The time constant is assumed to be zero for these plots

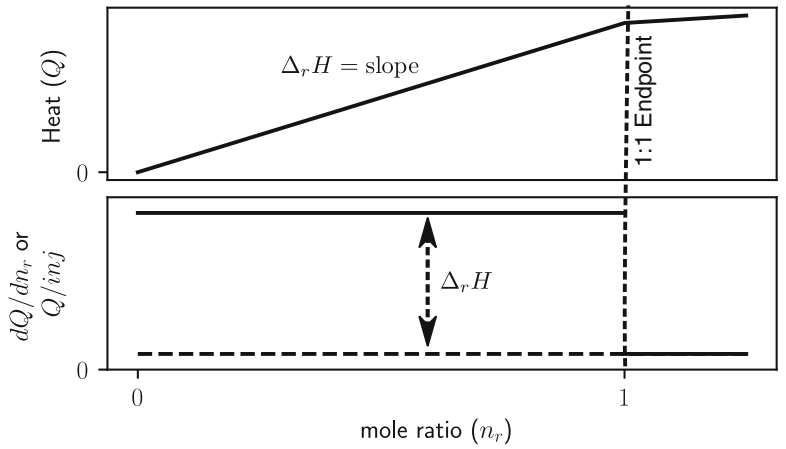

Fig. 1.2 Schematic plots of total heat to any point in the titration, *Q*, and of heat per increment of titrant, *dQ/dn*, versus the ratio of moles of titrant added to moles of titrate in the reaction vessel for a quantitative chemical reaction

exothermic reaction. The enthalpy change ($\Delta_r H$) is defined to be negative for exothermic reactions in both systems.

Reactions in a calorimeter can be initiated by changing temperature, pressure, or concentration. Changing concentration includes mixing reactants inside the calorimeter reaction vessel and inserting a reaction mixture into the reaction vessel. Temperature-scanning calorimeters are commonly referred to as "differential scanning calorimeters" or DSCs; no common mnemonic has been attached to pressure-scanning calorimeters, and concentration-scanning calorimeters are referred to as "titration calorimeters" or ITCs.

Heat exchange with the surroundings must be taken into account with all three calorimetric methods. Maintaining the surroundings at exactly the same temperature as the reaction vessel, i.e., adiabatic conditions, would avoid this, but adiabatic control is difficult, costly, and imperfect, so most calorimeters operate with surroundings at a controlled constant temperature, i.e., with isothermal surroundings. (Isoperibol is sometimes used to describe surroundings as having both constant temperature and constant pressure.) The method for correction for heat exchange with the surroundings in temperature change calorimetry is based on Newton's law of cooling, and procedures are well-known and documented (Hansen and Hart 2004). Correction for heat exchange with the surroundings in heat conduction and power compensation calorimeters is instrument specific and is done by calibrating the value of the calorimetric constant with a standard chemical reaction or with an accurate electrical heater that mimics the heat from a reaction. The heater used for power compensation usually does not accurately mimic the placement of heat from a chemical reaction, and thus the heat exchange with the surroundings differs.

Accurate calibration of the calorimetric constant (the constant used to convert the measured signal, usually voltage, electrical current, or electrical power) into heat or a

Table 1.2 Chemical reactions with well-known enthalpy changes. Data apply at infinite dilution and 25 °C. For a complete list of $\Delta_r H$ values for reactions of pH buffers at other concentrations and temperatures, see Goldberg et al. (2002)

Reaction	Enthalpy change, $\Delta_r H$/kJ mol^{-1}	Log_{10} equilibrium constant, K
$H^+(aq) + OH^-(aq) = H_2O(l)$	−55.77	13.997
$H^+(aq) + TRIS(aq) = HTRIS^+(aq)$	−47.45	8.072
$H^+(aq) + CO_3^{2-}(aq) = HCO_3^-$	−14.70	10.329
$H^+(aq) + HCO_3^-(aq) = CO_2(aq) + H_2O(l)$	−9.15	6.351

Table 1.3 Variables to consider in choosing a calorimeter (Hansen and Russell 2006)

1. Property to be measured, i.e., enthalpy change, equilibrium constant, heat capacity, etc.
2. Isothermal or temperature scanning
3. Temperature range
4. Pressure range
5. Reactant and product phases, i.e., liquid, solid, or gas and vapor pressures
6. Estimated total heat of reaction
7. Estimated rate of heat production
8. Sample availability
9. Desired sample size
10. Sample properties such as viscosity, solubility, etc.
11. Expected number of experiments per day

heat rate is obviously critical for obtaining accurate results. The reference standard is an electrical heater, but because of possible gains or losses of heat through thermal connections with the surroundings, calibration with an electrical heater should always be checked with a standard reaction that duplicates as closely as possible the reaction under study. Some reactions that can be used for this purpose are listed in Table 1.2. The reaction of Ca^{2+} with EDTA has recently been suggested as an alternative for this purpose, but the reaction is pH and buffer dependent and is thus not suggested for calibration of the calorimetric constant.

Many different designs of calorimeters for various purposes have been used beginning with the ice calorimeter (technically, a phase change compensation, isothermal, constant pressure calorimeter) Antoine Lavoisier used to study the respiratory heat rate produced by a guinea pig (Lavoisier and LaPlace 1780). The variables to be considered in designing or choosing a calorimeter are listed in Table 1.3.

Chapter 2
Introduction to Titration Calorimetry

Titration calorimetry is a relatively rapid way of obtaining thermodynamic data on reactions in solution. Enthalpy changes for solution of solids, for sorption of solutes on suspensions of sorbents, for reactions of gases with solutes, and for mixing of liquids and solutions can all be done in calorimeters equipped to handle gases, liquids, and solids, e.g., see Russell et al. (2006). However, this brief is largely limited to consideration of methods involving titration of a solution of one reactant into a solution of a second reactant. Titration calorimetry has three applications, analytical determinations of concentrations of reactants in solution, determination of enthalpy changes for reactions in solution, and under certain conditions, simultaneous determination of equilibrium constants and enthalpy changes for reactions in solution. This last application provides a full complement of thermodynamics for reactions in solution, i.e., the Gibbs energy change ($\Delta_r G = -RT\ln K$), the enthalpy change ($\Delta_r H$), and the entropy change ($\Delta_r S = (\Delta_r H - \Delta_r G)/T$); R is the gas constant, and T is the Kelvin temperature.

Addition of titrant to the reaction vessel can be done either by continuous addition of titrant, usually at a constant rate, or titrant can be added in increments. Because of the very short time constant of most temperature change calorimeters, continuous titrant addition can be done in these calorimeters without correction for the time constant. Continuous addition in heat conduction and power compensation calorimeters requires correction to account for the relatively long response time of these calorimeters, so incremental titrant addition has typically been used in these calorimeters. However, continuous titration can be done accurately in heat conduction and power compensation calorimeters if the time constant is treated as a parameter that is determined and corrected for during data processing and if the rate of titrant addition is optimized for the rate of reaction. Such a facility is implemented in the NanoAnalyze software package available from TA Instruments and in some other similar software packages. To make such a correction, the time constant for the rate of addition of titrant must be >6 times the time constant of the calorimeter, but continuous titrations are still much less time-consuming than incremental titrations.

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The other advantage to continuous titration over incremental titration is that many more data points are obtained, giving better resolution of the titration curve.

Partially filled reaction vessels are typically used in calorimeters with larger, i.e., >5 mL, reaction vessels, while overfilled vessels are used with small vessels, i.e., <2 mL. The change in solution volume during titrant addition to small, partially filled vessels can cause changes in stirring energy and heat losses to surroundings (Hansen et al. 1975), an effect that is usually negligible in larger vessels. Thus, small vessels are usually overfilled to avoid these problems. When titrant is added to an overfilled vessel, some of the solution is expelled during titrant addition. The composition of the expelled solution is necessary for calculating the composition of the remaining solution. However, the composition depends on mixing in the reaction vessel which varies with titrant and titrate densities, stirrer speed, and shape of the reaction vessel. Since the boundary between the reaction vessel and expelled solution is not well-defined, the active volume of overfilled reaction vessels must be calibrated. A reaction vessel volume is typically supplied by manufacturers as determined from the vessel dimensions, but note that this volume is not the same as the "active" vessel volume which must be calibrated by chemical reaction.

Titrant temperature must be controlled to be the same as titrate temperature, or a correction for the temperature difference must be made. To minimize both the effect of titrant temperature and uncertainty about the composition of expelled solution from overfilled reaction vessels, the volume of titrant is typically <10% of the volume of solution in the reaction vessel. Calibration of the buret for either incremental volume or rate of delivery for continuous titration is best done by weighing the amount of water delivered. However, since this procedure is not always possible, it can be done with a reaction with known stoichiometry, but the reaction must be free from interfering reactions. For example, the endpoint of a titration of a NaOH solution with an HCl solution is complicated by the presence of carbonate in the NaOH solution and carbonic acid (CO_2) in the HCl solution. Acid-base, complexation, and redox reactions suitable for buret calibration are titration of $KHCO_3$ solution with either a solution of HCl or a freshly prepared solution of sulfamic acid, titration of a solution of Ca^{2+} with an EDTA solution, and titration of a solution of Fe^{2+} or $Fe(CN)_6^{4-}$ with $K_2Cr_2O_7$ or Ce(IV) solution. Note that the concentrations and volumes of both solutions must be known with sufficient accuracy.

Mixing of the solution in the reaction vessel is typically done with a rotary stirrer which does work on the solution and thus introduces heat into the solution. Since the rate of heat input depends on the speed, choice of stirrer speed is a compromise between heat rate and mixing time. Stratification and slow or little mixing can occur with titrant and titrate solutions of significantly different densities. Stirrers are typically run at a constant speed which presumably introduces a constant rate of heat input. But, heat input rate also depends on solution viscosity which can change during a titration.

Mixing solutions of differing composition can produce a significant heat effect referred to as the heat of dilution or heat of mixing. Therefore, blank titrations of titrant into titrate media and of titrant media into titrate should be done to correct for such effects. These titrations can also serve as negative controls. Titration of an

unreactive liquid into the same liquid, e.g., a titration of water into water, should be done to determine the instrument baseline and as a test for any artifacts from an instrument malfunction or contamination.

Complete discussions of calibration of titration calorimeters are given in Hansen et al. (2011b) and Demarse et al. (2011). The calorimetric constant, injection volume or rate, and the active cell volume must all be calibrated. After calibration of the calorimeter and correction for all of the extraneous heat effects, the enthalpy change, $\Delta_r H$, for quantitative reactions, i.e., reactions that go to 99.9% completion, can be calculated simply by dividing the measured heat effect by the amount of reaction. $\Delta_r H$ can be evaluated for an incomplete reaction if the equilibrium constant is known by calculating the amount of reaction from the known solution composition.

Chapter 3
Determination of Equilibrium Constants by Titration Calorimetry

3.1 Introduction to Chemical Equilibrium

The equilibrium constant for a reaction

$$aA + bB + cC + \ldots = dD + eE + fF + \ldots \tag{3.1}$$

is defined as

$$K = \left([D]^d[E]^e[F]^f \ldots\right) / \left([A]^a[B]^b[C]^c \ldots\right), \text{i.e. products/reactants.} \tag{3.2}$$

The lowercase letters are the coefficients in the balanced reaction, uppercase letters represent the chemical species, brackets indicate concentrations or activities, and K is a constant under a given set of conditions, e.g., temperature and ionic strength. As an example, the reaction of EDTA with calcium ion in aqueous solution is written as

$$H_2EDTA^{2-}(aq) + Ca^{2+}(aq) = CaEDTA^{2-}(aq) + 2H^+(aq) \tag{3.3}$$

(aq) indicates the species are in aqueous solution, and the equilibrium constant expression is

$$K = \frac{\left[CaEDTA^{2-}\right][H^+]^2}{\left[H_2EDTA^{2-}\right]\left[Ca^{2+}\right]}. \tag{3.4}$$

The reactions and equilibrium constant expressions for two ligands (L) or metal ions binding with a macromolecule (M) with two binding sites are written as

$$L + M = LM \quad K_1 = [LM]/[L][M] \tag{3.5}$$
$$L + LM = L_2M \quad K_2 = [L_2M]/[L][LM] \tag{3.6}$$

or

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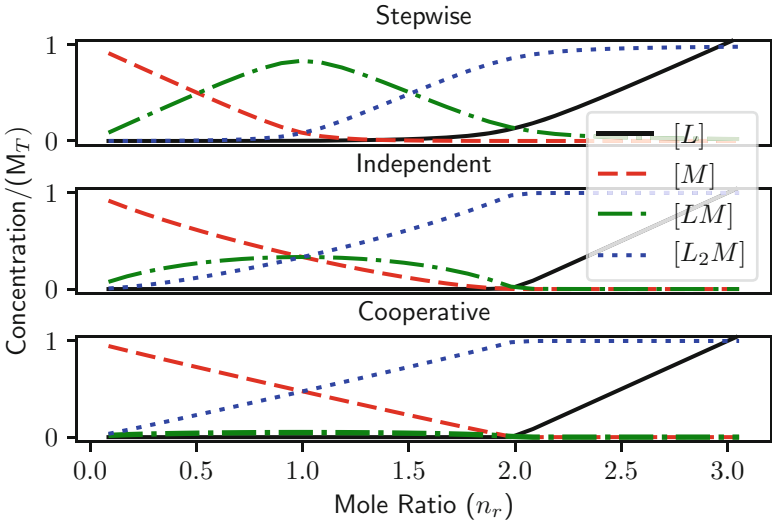

Fig. 3.1 Species distributions for reactions where two ligands bind to a central ion or molecule in stepwise, independent, and cooperative reactions

$$2L + M = L_2M \quad \beta_2 = K_1K_2 = [L_2M]/[L]^2[M]. \tag{3.7}$$

With two binding sites, three conditions are possible

$$K_1 > K_2 \quad \text{“stepwise”} \tag{3.8}$$
$$K_1 = K_2 \quad \text{“independent”} \tag{3.9}$$
$$K_1 < K_2 \quad \text{“cooperative”} \tag{3.10}$$

In the third case, K_1 cannot be accurately determined because [LM] may not be significant at any time during the titration. In this case, the equations become mathematically indeterminate, the system can only be accurately described by reaction 3.7, and only the product, K_1K_2 or β_2, can be determined.

The equilibrium concentrations of all species in any reaction system can be calculated if all the K values and the total concentrations of reactants are known. For example, in the L + M case, the mass balance equations are

$$[L]\text{total} = [L] + [LM] + 2[L_2M] \tag{3.11}$$
$$[M]\text{total} = [M] + [LM] + [L_2M]. \tag{3.12}$$

Species distributions for stepwise, independent, and cooperative cases for reactions 3.5 and 3.6 are shown in Fig. 3.1.

Note that the concentrations of solvents and pure solids and liquids are set equal to 1 and thus usually do not appear in the equilibrium constant expressions. For example, the equilibrium constant for reaction of Ba^{2+}(aq) with SO_4^{2-}(aq) to give $BaSO_4$(s) is written as

$$K = 1/\left[\mathrm{Ba}^{2+}(\mathrm{aq})\right]\left[\mathrm{SO_4}^{2-}(\mathrm{aq})\right], \tag{3.13}$$

and the equilibrium constant for reaction of $H^+(aq)$ with $OH^-(aq)$ to give H_2O is written as

$$K = 1/[\mathrm{H}^+(\mathrm{aq})][\mathrm{OH}^-(\mathrm{aq})]. \tag{3.14}$$

3.2 Analysis of Calorimetric Titration Data for Concentrations of Reactants

Titration calorimetry has roots in analytical methods and can be used to determine concentrations of reactants. One of these methods, thermometric or calorimetric titration, obtains concentrations from the endpoints in titrations as indicated by a change in heat production. If the reaction has a well-defined endpoint, n can be obtained from the amount of titrant added and endpoints in the titration curve. Figures 1.2, 3.3, 3.4, 3.5, 3.6, and 3.7 show examples of titration curves with endpoints that can be used to obtain stoichiometry. Endpoints are best observed in plots of total heat or heat per injection versus the total amount of titrant added. Endpoint sharpness depends on the product of K and the concentration of reactants, i.e., on KC, as shown in Fig. 3.2. As KC increases, endpoint sharpness increases. When an endpoint or endpoints are visible in a calorimetric titration curve, the reactions that are occurring and approximate $\Delta_r H$ values can often be deduced directly by visual inspection of the curve. Figures 3.3, 3.4, 3.5, 3.6, and 3.7 show

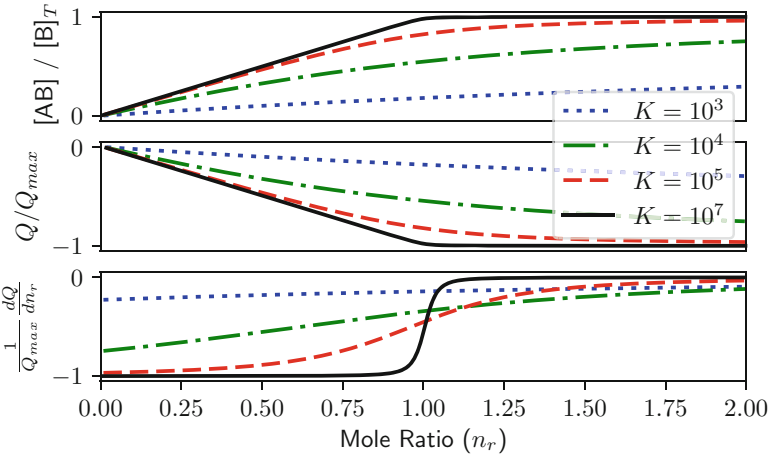

Fig. 3.2 Species distributions and plots of total heat, Q, and heat per increment of titrant, dQ/dn, for 1:1 reactions with differing equilibrium constants, K

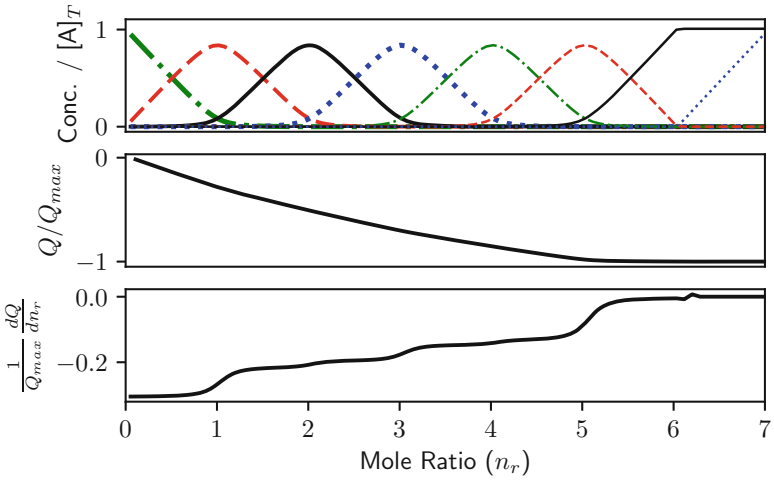

Fig. 3.3 Species distribution and titration curves for a stepwise reaction, i.e., $K_1 > K_2 > K_3 > \ldots$, with multiple binding sites, $A + xB = AB_x$, where x is an integer equal to 6, showing multiple endpoints. The total heat curve is a series of linear segments with a change in slope at the intersections

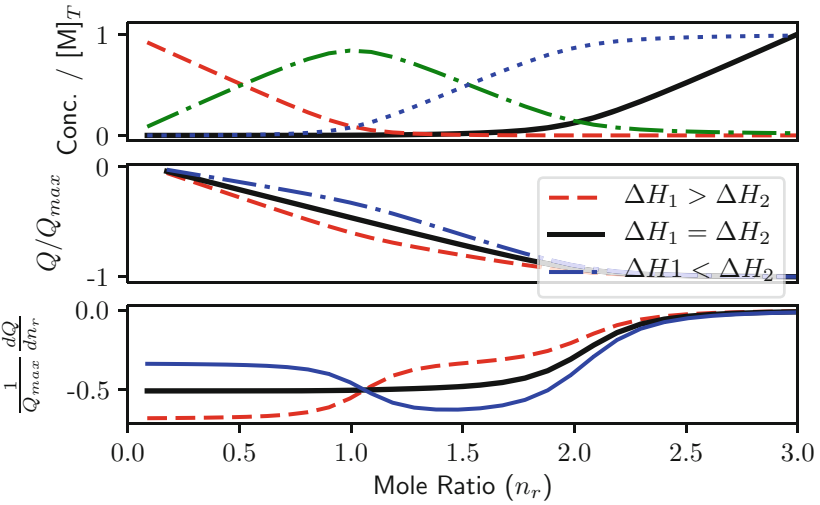

Fig. 3.4 Species distribution and titration curves for a stepwise ($K_1 > K_2$) 1:2 reaction, $A + B = AB$ and $AB + B = AB_2$, showing the effect of the difference in $\Delta_r H$ values on the endpoint between the two steps

examples of commonly encountered stoichiometries. Most of the current software for analysis of data from titration calorimetry has a modeling facility; you should try modeling reactions you are interested in to see what shape of titration curve those would give.

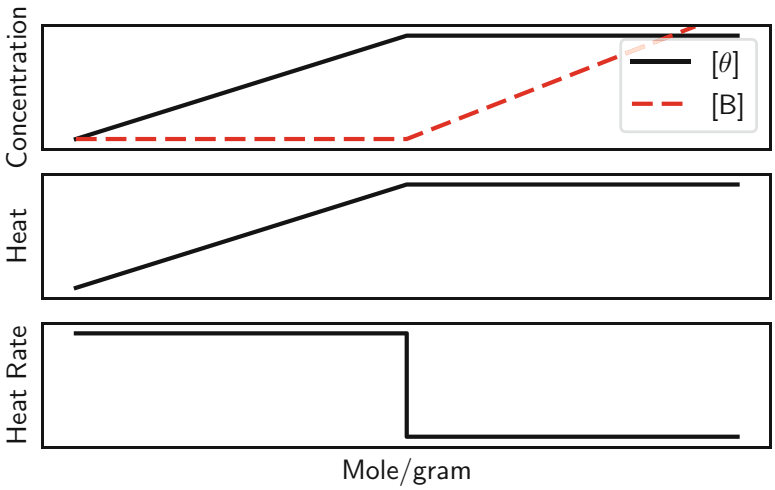

Fig. 3.5 Titration of a solution of a soluble molecule into a suspension of a solid sorbent

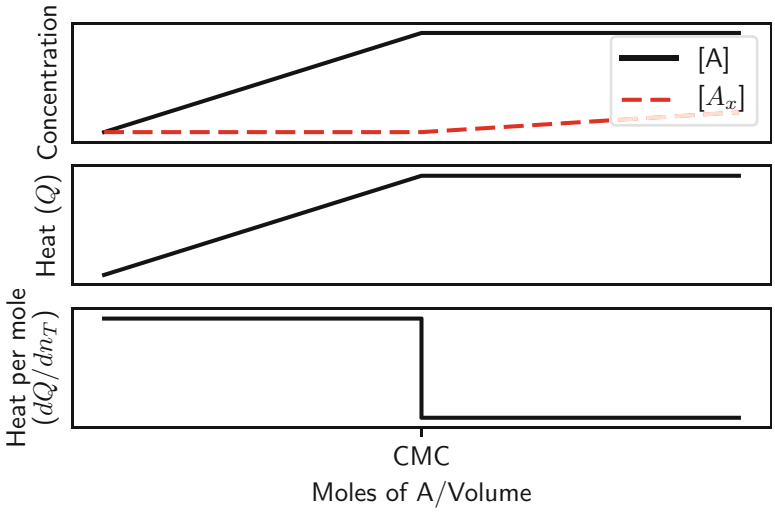

Fig. 3.6 A self-association or aggregation reaction, $A_x = xA$, where x is an integer. Such a reaction is run with a concentrated solution of A as the titrant. If x is small, it is modeled as a cooperative reaction, i.e., $K_{\text{assoc}} = [A_x]/[A]^x$, but when x is large, it is modeled as a phase change that occurs at a critical concentration of the aggregate, i.e., $K = [A]$ in equilibrium with the A_x phase

3.3 Analysis of Calorimetric Titration Data for Reaction Enthalpy Change, $\Delta_r H$

The enthalpy change for a reaction is defined as

$$\Delta_r H = Q/n. \tag{3.15}$$

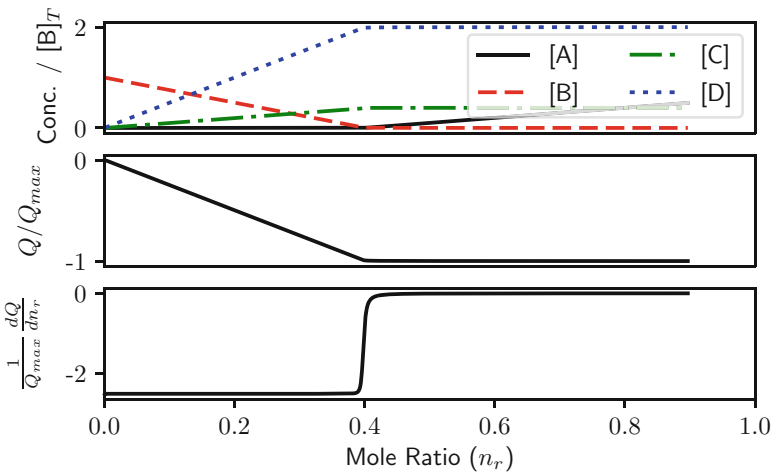

Fig. 3.7 A reaction with coefficients on titrant and reactant both >1, $xA + yB = zC + wD + \ldots$. Redox reactions commonly have this type of stoichiometry, e.g., $2MnO_4^- + 5H_2C_2O_4 + 6H^+ = 2Mn^{2+} + 10CO_2 + 8H_2O$, although some ligand-binding reactions also have this type of stoichiometry

Q is the amount of heat produced by n amount of reaction. The amount of reaction is in moles or in grams in cases where a molecular mass is not known or not applicable. Equation 3.15 is the basis for another method for the determination of a reactant known as injection enthalpimetry. The determination consists of injecting an excess of titrant into a solution containing the reactant (or injecting a known volume of the unknown into an excess of the other reactant) and measuring the heat produced, Q. The value of n can then be calculated with Eq. 3.15 if $\Delta_r H$ is known or can be determined with an experiment with a standard solution of the reactant; see Fig. 3.8.

3.4 Analysis of Calorimetric Titration Data for Simultaneous Determination of Equilibrium Constants (K), Enthalpy Changes ($\Delta_r H$), and Stoichiometry (n)

Simultaneous determination of equilibrium constants and enthalpy changes for incomplete reactions is possible under certain conditions. Analysis of titration data for simultaneous determination of equilibrium constants and enthalpy changes is done by combining a reaction model with mass balance equations and equations for the enthalpy changes for each reaction in the system and fitting this set of equations to the titration data. For example, for a 1:1 reaction, the reaction is

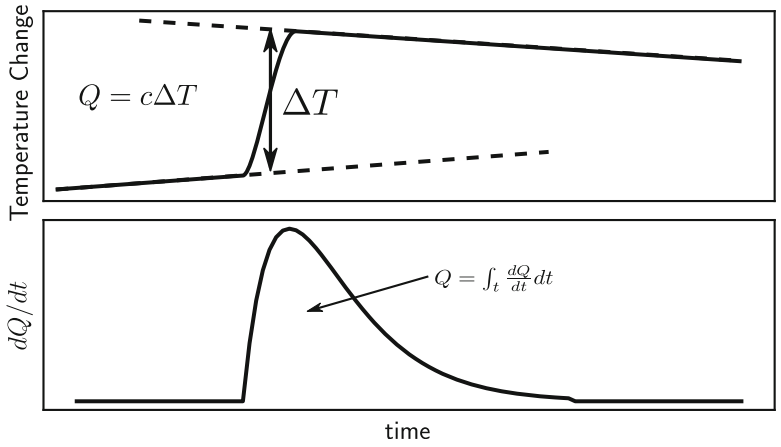

Fig. 3.8 Shows how total heat is calculated from addition of an excess of titrant to determine the amount of reactant present

$$A + B = AB. \tag{3.16}$$

The formation constant is

$$K = [AB]_i / [A]_i [B]_i. \tag{3.17}$$

Note that although K is constant, the values of $[AB]_i$, $[A]_i$, and $[B]_i$ vary through the titration and the subscript i indicates the data point number.

There are two mass balance equations for this system

$$[A\text{total}]_i = [A]_i + [AB]_i \tag{3.18}$$

and

$$[B\text{total}]_i = [B]_i + [AB]_i. \tag{3.19}$$

Note that the total concentrations of both A and B vary during the titration as titrant is added and the reactant is diluted. The equation for the total amount of heat produced to any point in the titration is

$$Q_i = \Delta_r H [AB]_i V_{\text{cell}} \tag{3.20}$$

Combining Eqs. 3.17, 3.18, 3.19, and 3.20 gives

$$Q_i = -\frac{\left(V^2 [A\text{total}]_i [B\text{total}]_i\right) \Delta_r H^2}{Q_i} + \left([A\text{total}]_i + [B\text{total}]_i\right) V \Delta_r H + \frac{V \Delta_r H}{K} \tag{3.21}$$

This equation has two unknown constants, K and $\Delta_r H$, and the equation cannot be solved with only one data point. But because several data points are collected in the titration, the system is overdetermined and the best values of K and $\Delta_r H$ are obtained from fitting all the data points in the titration curve simultaneously (See Chap. 5).

The same procedure and similar equations can be used to obtain K and $\Delta_r H$ values for all the reactions in systems with multiple reactions, but the algebra rapidly becomes complex, so software programs have been developed for this purpose, e.g., the NanoAnalyze program from TA Instruments. Some of these programs have several built-in models, and some allow the user to define a set of reactions to model the system.

3.5 Conditions for Simultaneous Determination of K, $\Delta_r H$, and n

The calorimetric method for the determination of K values is limited to nonquantitative reactions, or if there are multiple reactions in a system, the ratio of the K values for successive reactions must be sufficiently small to give a rounded endpoint between the reactions. If the reaction is quantitative or the ratio of K values is too large, Q becomes independent of K, and the system of equations becomes indeterminate. The extent of completion at the equivalence point can be estimated from how sharp the endpoint is as shown in Fig. 3.2. At the equivalence point in a 1:1 reaction where titrant A is added to a reactant B, the moles of titrant added equal the moles of reactant in the reaction vessel, and the total concentrations (C) of A and B are equal. Therefore

$$C_A = C_B = C. \tag{3.22}$$

Defining α as the fraction reacted gives the equilibrium concentrations of A and B as

$$[A] = [B] = (1 - \alpha)C. \tag{3.23}$$

Substitution into the equilibrium constant expression gives an equation that can be used to establish conditions that allow accurate determinations of equilibrium constants.

$$K = [AB]/[A][B] = \alpha C/(1-\alpha)^2 C^2 = \alpha/(1-\alpha)^2 C \text{ or rearranging, } KC = \alpha/(1-\alpha)^2. \tag{3.24}$$

Calculations and experiments show that if $50 < KC < 500$, the uncertainty in the determined equilibrium constant is minimal. Note that KC is called the "c parameter" in some literature (Wiseman et al. 1989; Broecker et al. 2011). The uncertainty in the equilibrium constant increases rapidly outside this range. Note that K is a

fundamental property of the reaction and not a variable, and thus C must be changed to adjust the value of KC for a given reaction.

The range of conditions suitable for determining equilibrium constants also depends on the $\Delta_r H$ value for the reaction, the volume of the reaction vessel, and the detection limit of the calorimeter, δQ (Hansen et al. 2011b). The total heat produced to the equivalence point is

$$Q_{ep} = -\Delta_r H V \alpha C. \tag{3.25}$$

V is the reaction vessel volume. Solving for C and substituting into Eq. 3.24

$$K/\mid \Delta_r H \mid = V\alpha^2/\left\{\left|Q_{ep}\right|(1-\alpha)^2\right\}. \tag{3.26}$$

If we assume the error in each data point, δQ is <0.01 $\mid Q_{ep} \mid$

$$K/\mid \Delta_r H \mid = V/\delta Q. \tag{3.27}$$

Since K and $\Delta_r H$ are properties of the reaction and V and δQ are properties of the calorimeter, we can use the inequality $KC < 500$ where $\alpha = 0.956$ (see Eq. 3.24) to obtain

$$\frac{K}{|\Delta_r H|} < 4.72 V/\delta Q \tag{3.28}$$

as an upper limit for $K/\mid \Delta H \mid$ values that can be determined in a calorimeter with a reaction vessel volume, V, and a detection limit, δQ. Using the inequality $KC > 50$ where $\alpha = 0.868$ (see Eq. 3.24) an equation for the lower limit of $K/\mid \Delta_r H \mid$ values that can be obtained.

$$\frac{K}{|\Delta_r H|} > 0.43 V/\delta Q. \tag{3.29}$$

Figure 3.9 shows the limits obtained from these inequalities for a 1:1 reaction.

3.6 General Procedure for Simultaneous Determination of K, $\Delta_r H$, and n

Simultaneous determination of K and $\Delta_r H$ from titration calorimetric data always involves the following series of steps in the process.

1. Selection of a reaction model consisting of balanced chemical reactions.
2. Estimating starting values for K, $\Delta_r H$, and n for each of the reactions in the model.
3. Calculating a species distribution from the estimated K values, reactant concentration, titrant concentration, cell volume, and buret delivery volume.

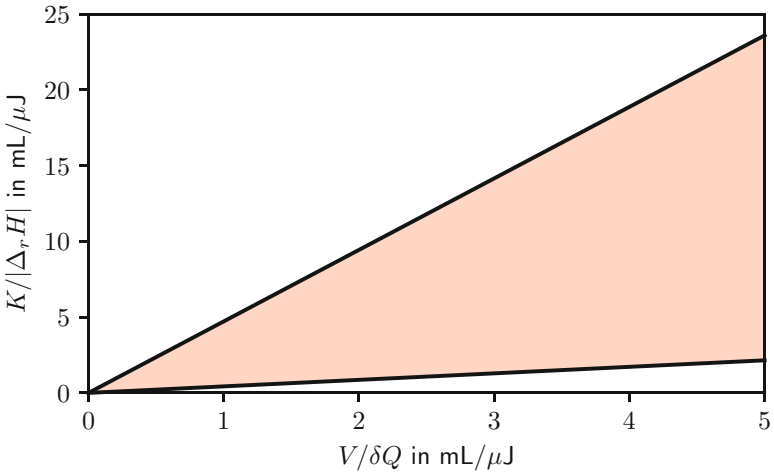

Fig. 3.9 Upper and lower limits for the ratio $K/|\Delta_r H|$ in simultaneous determination of the equilibrium constant and enthalpy change as a function of the reaction vessel volume and heat detection limit of the calorimeter (Hansen et al. 2011b)

4. Calculating the expected titration curve from the species distribution and $\Delta_r H$ values for each of the reactions in the model. Uncertain stoichiometry, n, and the blank and baseline corrections may be adjusted in this step. If the ionic strength varies significantly during the titration, correction for activity coefficients may be introduced in this step.
5. Comparison of the calculated titration curve with the observed titration curve.
6. Estimation of better values for n, K, $\Delta_r H$ and baseline values.
7. Iteration of steps 3–6 until a best fit is obtained.

Depending on the software, step 1 may be the only input required from the human operator. Thus, the operator needs to carefully check to make sure that the results from the fit make sense. First, does the model actually fit the data? Second, do the n, K, and $\Delta_r H$ values make chemical sense? Third, are the fitted variables truly independent or are some of these highly correlated so that uncertainties are large? If the answer to either or both of the first and second questions is "no," a different model needs to be considered.

3.7 Temperature Dependencies of K and $\Delta_r H$

The temperature dependence of K is given by

$$\left(\frac{d \ln K}{dT}\right) = \Delta_r H / RT^2. \tag{3.30}$$

The temperature dependence of $\Delta_r H$ is given by

$$\left(\frac{d\Delta_r H}{dT}\right) = \Delta_r C_p. \tag{3.31}$$

R is the gas constant, T is Kelvin temperature, and $\Delta_r C_p$ is the change in constant pressure heat capacity of the reaction. If $\Delta_r C_p$ is not constant over the temperature range, the temperature dependence can be described by

$$\Delta_r C_p = a + bT + c/T^2. \tag{3.32}$$

3.8 Choosing a Reaction Model

The method for simultaneous determination of equilibrium constants and enthalpy changes can be generalized to any number of reactions by writing all of the reactions that occur in the system during the titration, writing an equilibrium constant expression for each of the reactions, writing a mass balance equation for each of the reactants, writing equations for the heat produced by each of the reactions, combining all of the equations algebraically into a single expression, and fitting the expression to the titration data. However, doing the algebra for a system with more than two reactions is extremely complex. Therefore, a facility for doing all but writing the reactions has been implemented as part of the data analysis software produced by some manufacturers and independents. But it must be left to the user to select the right set of reactions to fit the data. Choosing reactions that do not correctly describe the chemistry will either result in a poor fit to the model or a good fit to the model but with incorrect K and $\Delta_r H$ values. In the latter case, comparison with analogous reactions with known K and $\Delta_r H$ values is very helpful. Also, note that K and $\Delta_r H$ should not vary with concentration, so running experiments with differing total concentrations is a good way to validate a model.

Oftentimes the chemical reactions occurring during the titration are unknown, so choosing the correct reaction model must be done from information contained in the titration curve or from other data. In determining a reaction model from a titration curve, the first thing to ask is "Are there endpoints present in the titration curve?" If so, the stoichiometry of the reactions can be obtained from the curve. Figures 1.2, 3.3, 3.4, 3.5, 3.6, and 3.7 indicate how this is done. If no endpoints are present, it may be possible to increase the concentrations of reagents and obtain a curve with discernable endpoints; see Sect. 3.5. If it is not possible to obtain a curve with endpoints, the reaction stoichiometry must be determined by another method since such a curve is fit equally well by the reaction $A + xB = AB_x$ with any positive integer value of x or other possible stoichiometries. A full validation of a model requires that titrations be run at different concentrations of titrant and reactant to show that all K and $\Delta_r H$ values are independent of concentration. Since non-specific binding with a buffer or other components of the solvent can also affect the K and $\Delta_r H$ values, all

other components of the solutions must be kept constant. A reverse titration, i.e., switching titrant and reactant, is also helpful in model validation. The reverse titration should be considered for use when a titration yields unexpected stoichiometry or when there is concern for one of the concentrations of the components. Typically, the forward titration is done with the ligand, *L*, in the syringe and reactant with multiple binding sites, *M*, in the cell. At the beginning of the forward titration, the ratio of ligand to reactant is low, and the 1:1 species predominates. At the beginning of the reverse titration, higher-order species predominate, i.e., ML_n, followed by conversion of this species to a lower order complex, ML_{n-1}. Fitting a model to the forward and reverse titrations should result in the same equilibrium constants and $\Delta_r H$ values. Failure to obtain the same K and $\Delta_r H$ values indicates either an incorrect concentration or a wrong model for the reactions. In any case, the values of equilibrium constants and enthalpy changes obtained from fitting the curve must be subjected to a "truth" test, i.e., given the chemistry, do the numbers make sense? (see Sect. 3.9).

3.9 Some General Rules for Estimating K and $\Delta_r H$ for Reactions

$\Delta_r H$ values exhibit many regularities that can be used to estimate an unknown value for a reaction. For example, $\Delta_r H$ for reaction of any organic compound or material with O_2 to produce water and CO_2 as the major products is approximately −455 kJ/mole of O_2. This value is essentially independent of the structure of the organic compound or material. $\Delta_r H$ values for acid-base reactions of organic acids in water range from 0 to 60 kJ/mole and depend almost entirely on the acid-base functional group and not on the structure of the remainder of the molecule, e.g., $\Delta_r H$ for ionization of carboxylic acids is ≈0 kJ/mole (Christensen et al. 1967) and $\Delta_r H$ for ionization of alkylammonium ions is ≈50 kJ/mole (Christensen et al. 1969). Metal-ligand binding and binding of polar compounds to macromolecules exhibit similar regularities in $\Delta_r H$ values. Non-covalent interactions such as hydrogen bonds typically have $\Delta_r H$ values less than 5 kJ/mole for dissociation. Binding of inhibitors to the active site of enzymes generally has $\Delta_r H < 30$ kJ/mole for dissociation.

Although equilibrium constants vary much more than $\Delta_r H$ with molecular structure, regularities often exist as linear relations between the Gibbs energy change, $\Delta_r G$, the enthalpy change, $\Delta_r H$, and/or the entropy change, $\Delta_r S$. For example, for proton ionization from a given acid group, $\Delta_r H$ is typically constant, and since $\Delta_r G = \Delta_r H - T\Delta_r S$, a linear relation between $\Delta_r G$ and $\Delta_r S$ is expected. In aqueous solutions, another important regularity is compensation between $\Delta_r H$ and $\Delta_r S$ for non-covalent interactions of polar molecules that produce a near constant $\Delta_r G$ value for a series of related reactions.

3.10 Uncertainties in Results of Simultaneous Determination of K, $\Delta_r H$, and n

A common mistake made in considering the accuracy of simultaneous calorimetric determinations of K, $\Delta_r H$, and n is to report the precision or reproducibility of replicate determinations instead of the appropriate uncertainties. Because observed values of K and $\Delta_r H$ are correlated, i.e., increasing K decreases $\Delta_r H$ and vice versa. In assessing uncertainties, correlation of parameters must be taken into account along with random and systematic errors to obtain a valid estimate of the actual uncertainty in the values reported. (See Chap. 5 for a detailed discussion.)

Chapter 4
Determination of Reaction Kinetics by Calorimetry

4.1 Introduction to Chemical Kinetics

Calorimetry has been used to measure rates of reaction since the late 1700s when Antoine Lavoisier used an ice calorimeter to measure the rate of heat produced by a guinea pig (Lavoisier and LaPlace 1780). In proving that respiration was simply a slow combustion, Lavoisier also measured the rates of consumption of oxygen and production of CO_2. Lavoisier's experiments demonstrate many of the advantages of calorimetry for kinetic measurements; rates can be measured directly and noninvasively in any media. Measurements of rates instead of measuring the amount of product accumulated over time (or of reactant lost) are faster, simpler, and more sensitive than most other methods, particularly for slow reactions. Aside from methods that count radioactive decay rates, heat conduction and power compensation calorimetry are the only methods that measure rates directly. Calorimetry has been shown to be capable of measuring rates of reactions with half-lives greater than 1000 years (Hansen 1996). Calorimetry makes no requirements of the system except that it fits within the reaction vessel and does not react with any other materials in the reaction vessel. Systems can be gaseous, liquid, solid, or even a living organism, e.g., plant tissue, insects, microorganism cultures, and animals.

Heat conduction and power compensation calorimeters measure heat rates directly, and heat rates can be obtained as the slope of temperature versus time in temperature change calorimetry. Because calorimetry measures rates versus time, the rate law must usually be cast in terms of the heat rate, (dQ/dt), versus time, t, instead of the more familiar rate versus amount of reactant or product, n. In general,

$$\left(\frac{dQ}{dt}\right) = -\Delta_r H\left(\frac{dn}{dt}\right) = -\Delta_r Hk\{f(n)\} = -\Delta_r Hk\{g(t)\}. \tag{4.1}$$

For example, for a zero order reaction,

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$$\left(\frac{dQ}{dt}\right) = -\Delta_r Hk, \tag{4.2}$$

and for a first-order reaction,

$$\left(\frac{dQ}{dt}\right) = -\Delta_r Hke^{-kt}, \tag{4.3}$$

Or, in linear form,

$$\ln\left(\frac{dQ}{dt}\right) = \ln\left(-\Delta_r Hk\right) - kt. \tag{4.4}$$

A plot of $\ln(dQ/dt)$ versus time has a slope of $-k$ and an intercept of $\ln(-\Delta_r Hk)$. Note that $\Delta_r H$ and k are in general not separable in the equations for heat rate as a function of time, and therefore only the product $\Delta_r Hk$ is available from fitting the rate law to the calorimetric data. However, $\Delta_r H$ can usually be determined from the measured total heat, see Fig. 3.8, and k can then be calculated from the product, $\Delta_r Hk$. Also note that k for a first-order reaction is the reciprocal of the time constant for the reaction.

The process for converting from $f(n)$ to $g(t)$ is illustrated with the following equations for a first-order reaction for which

$$\left(\frac{dn}{dt}\right) = -kn \tag{4.5}$$

$$\int dn/n = -k\int_0^t dt \tag{4.6}$$

$$\ln(n) = -kt \tag{4.7}$$

$$n = e^{-kt}. \tag{4.8}$$

Substitution of Eq. 4.8 into Eq. 4.5 gives

$$(dn/dt) = -ke^{-kt}. \tag{4.9}$$

The function $g(t)$ for other rate laws is given in Hansen et al. (1988, 2011a). The appropriate rate law, i.e., $g(t)$ in Eq. 4.1, to use in fitting a particular data set can usually be determined from the shape of the curve.

Just as in any other method, rates that can be measured calorimetrically depend on the time constant of the instrument. As a rule of thumb, the time constant of the reaction must be at least five times greater than the time constant of the calorimeter, see Table I in the introduction (Hansen et al. 2011a). Temperature change calorimetry typically has a time constant of a fraction of a second to a few seconds, so these calorimeters can be used for relatively fast reactions, e.g., see Tapscott et al. (1975). However, because heat loss corrections are necessary, and obtained by extrapolation of the baselines from before and after reaction, temperature change calorimetry is

limited to reactions that go to completion in 1–5 h. The lower the heat exchange rate, the longer the completion time allowed. Heat conduction and power compensation calorimeters have much longer time constants, on the order of minutes, and are thus limited to relatively slow reactions. But because these calorimeters do not require correction for heat exchange with the surroundings, they are suitable for measuring the kinetics of very slow reactions that may occur over days, weeks, months, or years (Hansen 2000).

For reactions that have a time constant near the calorimeter time constant, the Tian equation can be used to correct the data for the calorimeter time constant, τ.

$$\left(\frac{dQ}{dt}\right)\text{corrected} = \left(\frac{dQ}{dt}\right)\text{measured} + \tau\left(\frac{d^2Q}{dt^2}\right)\text{measured} \tag{4.10}$$

Note that the Tian equation can only be used if the calorimeter time constant is known and invariant with time and reaction vessel contents. However, in many cases, the time constant is unknown and varies with the contents of the reaction vessel. In this case, τ must be included as a fitting variable. Equations that include the time constant as a fitting parameter have been derived (Hansen et al. 2011a) for fitting first order, *n*th order, first order in each of two reactants, autocatalytic reactions, and Michaelis-Menten (Briggs-Haldane) mechanisms and are included in the NanoAnalyze program from TA Instruments.

4.2 Reactions with Rate Determined by a Catalyst

The kinetics of reactions with rate determined by a catalyst can be done either by injecting catalyst into a solution of reactant(s) or by injecting the reactant(s) into a solution of the catalyst. In either case, special care must be taken not to allow any mixing prior to injection. The choice of which method to use is usually determined by the physical properties of the reactant(s) and catalyst. Catalysts can be powdered solids, enzymes, membrane-bound enzymes, or a solution or suspension of a catalyst.

Two calorimetric methods have been used to obtain the kinetics of catalyzed reactions. The single injection method uses a single rapid injection of either the catalyst or substrate to start the reaction and then follows the heat rate over time, usually until the baseline heat rate is reached (Transtrum et al. 2015). In the multiple injection method, incremental injections of substrate are made, and the heat rate after each injection is measured (Di Trani et al. 2017; Hansen et al. 2016). Injections are usually made until the heat rate does not increase on injecting more reactant, indicating the catalyst is saturated. The multiple injection method requires that concentrations be adjusted within a narrow range to obtain good results. The ratio of concentration of catalyst to substrate must be low enough that heat rates after each injection are relatively constant and sufficiently low that saturation can be reached. The single injection method is typically faster, requires less material, and is less

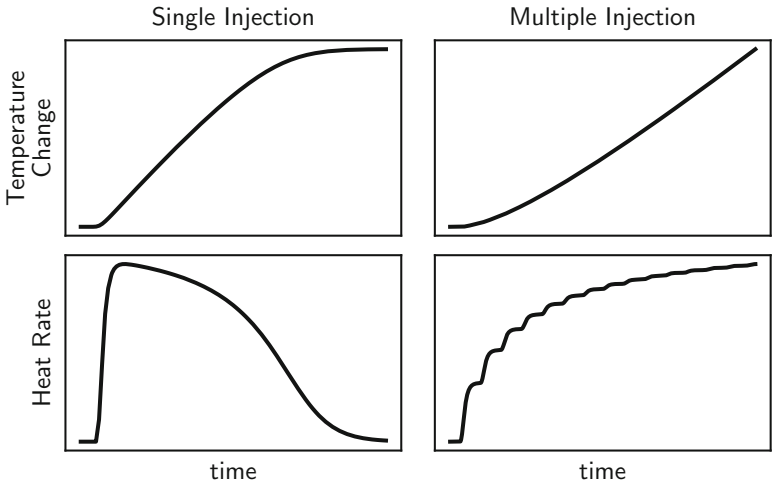

Fig. 4.1 The form of the data produced by the single injection and multiple injection methods for determination of reaction kinetics for a catalyzed reaction

sensitive to concentrations. Both methods require that enzyme saturation be reached in order to satisfy conditions for accurate fitting of the Michaelis-Menten (Briggs-Haldane) equation (Hansen et al. 2016). The form of the data from both methods is shown in Fig. 4.1.

4.3 Reactions with Rate Determined by Activities of Reactants

The kinetics of reactions with rate determined by activities of reactants can often be combined with a determination of the equilibrium constant and enthalpy change for the reaction. A good example is binding of a ligand to a protein. The kinetics of such a reaction should be determined as a function of the concentrations of both reactants followed by global fitting of all the data. This procedure is necessary to validate the choice of rate law used to describe the kinetics. An example of such a data set is given in Fig. 4.2.

4.4 Temperature Dependence of the Rate Constant

Once a rate law is established, the temperature dependence of the rate constant, k, can be determined by measuring the rate at several temperatures. An Arrhenius function

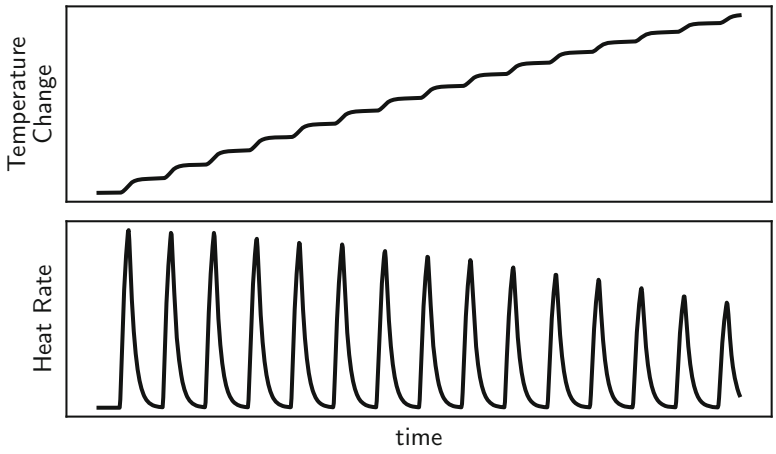

Fig. 4.2 Form of the data from titration of *A* into *B* with a slow reaction to form *AB* with a rate law, $d[AB]/dt = k[A][B]$

$$k = Ae^{-E_a/RT} \tag{4.11}$$

or in linear form

$$\ln k = \ln A - E_a/RT \tag{4.12}$$

is often used to describe the temperature dependence of the rate constant. A is the frequency factor, E_a is the activation energy, R is the gas constant, and T is the Kelvin temperature. A plot of ln k versus $1/T$ has a slope of $-E_a/R$ and an intercept of ln A.

An alternative is the Eyring-Polanyi formulation which is more complex. However, the parameters in the Eyring-Polanyi formulation are related to fundamental constants:

$$k = aT\exp\left(-\Delta G^{\ddagger}/RT\right) = aT\exp\left(-\Delta H^{\ddagger}/RT + \Delta S^{\ddagger}/R\right) \tag{4.13}$$

or in linear form

$$\ln k/T = -\Delta H^{\ddagger}/RT + \ln a + \Delta S^{\ddagger}/R. \tag{4.14}$$

$\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ are, respectively, the Gibbs energy, enthalpy, and entropy of activation, and a is a constant equal to $\kappa k_B/h$ where κ is a transmission coefficient usually set equal to one, k_B is Boltzmann's constant, and h is Planck's constant. A plot of $\ln k/T$ versus $1/T$ has a slope of $-\Delta H^{\ddagger}/R$ and an intercept of $(\ln a + \Delta S^{\ddagger}/R)$. This formulation is sometimes useful for describing more complex temperature dependencies than can be fit by the Arrhenius function but requires very accurate kinetic data to obtain significant values for $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$.

Chapter 5
Statistics of Curve Fitting

5.1 Introduction

In many cases, the goal of ITC measurements is to infer quantitative values for reaction parameters such as equilibrium constants, enthalpy changes, and rate constants. To do this, a mathematical model of the reaction(s) going on in the calorimeter is first constructed. Then the predictions of the model are compared against the experimental observations. Because the model includes several unknown parameters, it is necessary to vary the parameters until the model predictions are "as close as possible" to the observed data.

The primary challenge to parameter estimation alluded to throughout this brief is that data are generally not equally informative about all parameters (or combinations of parameters). For example, consider the case of a ligand binding cooperatively to a macromolecule with two binding sites with $K_1 < K_2$, where K_1 is indeterminate (see Fig. 3.1). In this case, K_1 cannot be estimated accurately because $[LM]$ is not significant at any time during the titration. To be more precise, an estimate of K_1 can always be obtained, but the numerical value obtained may not be close to the true value that generated the data. In the language of statistics, one would say that the parameter K_1 is practically unidentifiable. Because not all parameters may be accurately identified from data, the statistical confidence should always be reported together with parameter estimates.

The goals of this section are twofold. First, we demonstrate how one goes about obtaining a least squares estimate of parameters from calorimetric titration data. Second, we show how to statistically interpret these estimates, i.e., how to measure and assess the confidence in parameter values. The latter question is complicated by the existence of several schools in the statistics community that, in addition to differing in their methods, reflect centuries-old philosophical disagreements about what it means to perform statistical inference. These schools are generally known as frequentists and Bayesians; however, more recent information-theoretic approaches have also grown in popularity. These technicalities are not discussed here. Instead,

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our focus is on providing methods that are easy to both implement and understand for most calorimetric titration practitioners.

5.2 The Least Squares Estimator

Least squares estimates are by far the most common method of fitting calorimetric titration data and the method we use here. The least squares estimator can be applied to both integrated and kinetic data and will provide asymptotic confidence estimates almost automatically. It can also be adapted to the jackknife and bootstrap methods for estimating confidence in the inferred parameter values.

As a starting point, we assume one has a mathematical model for the total heat or heat rate measured by the calorimeter. The mathematical form of the model will depend on a number of details including the type of titration experiment, whether the data are integrated or differentiated, and what reactions are being modeled. In general, one does not have an explicit expression for the mathematical model but rather a computational algorithm for evaluating it.

In order to abstract away these technical details, we assume that the mathematical model can be represented as a function $y(t;\theta)$. In this expression, θ is a vector of parameters to be estimated from the data, while t is known as the independent variable. For the case of kinetic data, t is time, while if the data are from a titration, then t could be either injection number or mole ratio. The idea is that the function $y(t;\theta)$ will be evaluated for fixed parameter values at different values of t, denoted t_i. For each value of t_i, there is an associated observation d_i. The goal is to vary the values of the parameter vector θ until the predicted $y(t_i;\theta)$ are as close as possible to the values d_i.

To illustrate the relevant principles throughout this chapter, we consider a concrete example; the case of two ligands (L) binding to a macromolecule (M) with two binding sites for which we have observed integrated data after multiple injections. The governing equations for the equilibrium concentrations are given in Chap. 3. The parameters to be estimated are two equilibrium constants, K_1 and K_2, and two enthalpy changes, $\Delta_r H_1$ and $\Delta_r H_2$, for binding to the two sites. We therefore have that $\theta = (K_1, K_2, \Delta_r H_1, \Delta_r H_2)$. In practice, one may also fit several other parameters, such as the titrant or reactant concentration. Here we focus on these four parameters to illustrate the conceptual procedure and potential challenges that may arise. After each injection, the known total concentrations of ligand and macromolecule are combined with the equilibrium constants to determine the four concentrations $[L]$, $[M]$, $[LM]$, and $[L_2M]$. Finding these concentrations involves solving a set of four nonlinear equations. Once the concentration of each species is calculated after each injection, we can also calculate the amount of each of the reactions that has taken place after each injection and the associated heat for each injection. Although a closed form expression for this heat is not given, the point is that there exists a function $y(t;\theta)$ that makes quantitative predictions for the total heat observed after each injection and this function can be numerically evaluated.

To quantify the difference between the predictions of the model and the observed data, we construct the residuals:

$$r_i(\theta) = w_i[d_i - y(t_i;\theta)]. \tag{5.1}$$

The residuals are a collection of functions of the parameters. Notice that there is one residual for each observation, d_i. Equation 5.1 introduces optional weights, w_i. In many cases, it is assumed that $w_i = 1$ in which case the method is known as ordinary least squares (OLS). However, in some cases, one may wish to weight some data more than others for estimating parameters. In this case, different values of w_i are used, and the method is known as weighted least squares (WLS). The most common use for WLS is when experiments are repeated and error bars estimated for each observed data point. In this case, if the uncertainty associated with observation i is σ_i, then $w_i = 1/\sigma_i$.

Finally, from the residuals, we construct the chi-squared cost function:

$$\chi^2(\theta) = \sum_i r_i^2. \tag{5.2}$$

This is a single function of the parameters that summarizes the difference between all model predictions and experimental observations. The cost is a figure of merit that measures the statistical distance between the observed data and the model's predictions for a choice of parameter values. The goal is to vary the parameters (θ) until this function is as small as possible. The value of θ that minimizes the cost is known as *the least squares estimator*, and we denote it by $\hat{\theta}$. We will typically refer to $\hat{\theta}$ as the "best fit."

5.3 Finding the Best Fit

Finding the best fit can be a numerically challenging task. The cost is almost always a highly nonlinear function of multiple parameters, and there could be several local minima to this function. A more subtle but common problem is that the cost function typically has very broad "plateaus." That is, the cost may be very insensitive to changes in one or more parameters or combinations of parameters. The problem of constructing good algorithms for finding best fits is beyond the scope of this brief. Most algorithms require a user to provide an initial estimate of the parameter values. The importance of providing good initial estimates cannot be overstated. If poor initial estimates are provided, then the fitting algorithm may return a spurious solution by converging to a local minimum or mistaking a very flat region of the cost surface for a minimum. Best practices should always involve multiple fitting attempts with different values of the initial guess to make sure that the best fit has really been found.

5.4 The Meaning of Statistical Uncertainty

To illustrate the general procedure, we return to our example model of two binding sites. We have evaluated the model's predictions using "true" parameters $K_1 = 2 \times 10^7$ M^{-1} and $K_2 = 1 \times 10^8$ M^{-1} and $\Delta_r H_1 = 50$ kJ/mol and $\Delta_r H_2 = 25$ kJ/mol for a sequence of 31 injections. Since K_1 and K_2 are very nearly equal, this corresponds to the "independent" scenario in Fig. 3.1. To each of the model predictions, we next add a small random number to mimic measurement error. Finally, we pretend to not know the values of the true parameters and minimize the chi-squared cost. We find the best fit values to be $K_1 = 1.7 \times 10^7$, $K_2 = 8.3 \times 10^7$, $\Delta_r H_1 = 49.4$, and $\Delta_r H_2 = 25.56$ kJ/mole. Notice that we do not recover the "true" values, although our estimates are fairly close. Of course, the reason for the discrepancy is that we have added random numbers to our "observations." If we had not done this, we would have exactly recovered the "true" parameter values. On the other hand, if we had added different random numbers, our estimates would have similarly been different.

In general, one would hope that the best fit values are close to the true values, as they were for this example; however, this is not always the case. If we had repeated this exercise for the case of a "cooperative" binding, we would have recovered a much different scenario. In this case, it is difficult to estimate K_1 because there is never any significant fraction of $[LM]$. In fact, in this case, K_1 can become arbitrarily small, and the predictions of the model change only slightly. In this case, the estimated value of K_1 is very sensitive to small measurement noise.

This simple exercise illustrates the problem with simply reporting results of the fit. In general, one is interested in extracting (to the extent possible) the "true" values that gave rise to the data, but the least squares estimate is only an approximation of these values. The results are worthless unless accompanied by some measure of how close we believe this estimate is to the true values. Such a measure is known in statistics as a confidence interval.

5.5 Confidence Intervals

To explore this idea further, consider the following numerical experiment. We repeat the fitting procedure described before with different random numbers added to the model predictions. This generates a collection of "best fits" for different realizations of the measurement error. We are interested in characterizing the distribution of this set of best fits. This distribution lives in a four-dimensional parameter space, making it hard to visualize. In Fig. 5.1 we show the projection of this distribution onto each parameter axis. For each of the four parameters, the distribution of best fits is very nearly a Gaussian centered near the true value.

We can also project the four-dimensional distribution onto the two-dimensional space for each pair of parameters, as in Fig. 5.2. In this case, a more interesting

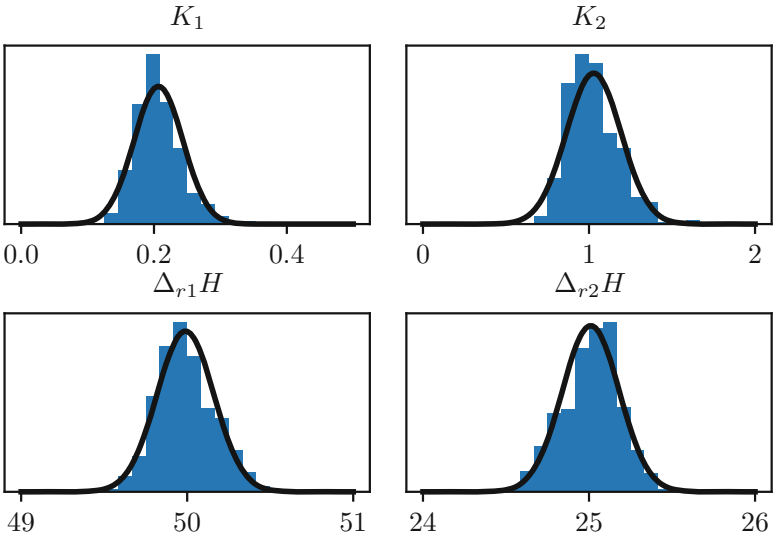

Fig. 5.1 Marginal distributions for best fit parameters for an ensemble of randomly generated data

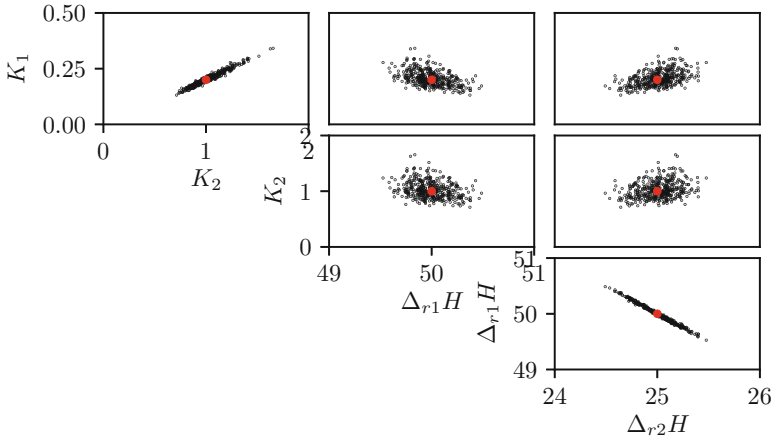

Fig. 5.2 Pairwise marginal distributions for best fit parameters

structure is observed. Here, the distributions are also very nearly Gaussian, though in two dimensions. In other words, the distribution of best fits are approximately ellipses in the two-dimensional subspaces. The best fit values are very close to the center of the ellipses.

5.6 Parameter Correlations

Notice that the axes of the ellipses do not align with any of the bare parameter axes. As an example, consider the long, narrow ellipse for the cross section of $\Delta_r H_1$ and $\Delta_r H_2$. The fact that this ellipse is very anisotropic shows that our estimates of these parameters are highly correlated. More precisely, these two parameters are negatively correlated because the orientation of the ellipse has a negative slope. The physical interpretation of this correlation is that the available measurements are very informative about the total amount of heat generated, but less informative about whether the heat came from the first or second reaction. Consequently, the model's predictions are approximately unchanged by increasing $\Delta_r H_1$ and decreasing $\Delta_r H_2$ by an appropriate amount so that the total heat generated is constant.

Similarly, the two equilibrium constants are strongly correlated in a positive manner. This means that the measurements are more informative about the ratio of the equilibrium constants than their product. The model predictions are left relatively unchanged if K_1 and K_2 are both increased or decreased by the same factor. This observation motivates the use of the β notation in Chap. 3. In contrast, there is very little correlation between the equilibrium constants and either of the heats. The distribution of points in each of these four cross sections is approximately circular, indicating that variation in one parameter cannot be compensated for by variation in the other.

This example illustrates the importance of not only estimating the uncertainty in individual parameters but the error correlation in those estimates. The correlation information can be conveniently summarized in a correlation matrix, corr(θ) (not to be confused with the similar covariance matrix introduced below). Given an ensemble of parameter estimates θ_i (for $i = 1 \ldots n$), the average value of these estimates, denoted by $\overline{\theta}$ is

$$\overline{\theta} = \frac{1}{n} \sum_{i=1}^{n} \theta_i, \tag{5.3}$$

where n is the number of samples in the ensemble. An unbiased estimate of the sample covariance matrix $\sum$ is

$$\Sigma = \frac{1}{n-1} \sum_{i=1}^{n} \left(\theta_i - \overline{\theta}\right)\left(\theta_i - \overline{\theta}\right)^T. \tag{5.4}$$

Finally, the correlation matrix is given by

$$\text{corr}(\theta) = \text{diag}(\Sigma)^{-\frac{1}{2}}\, \Sigma\, \text{diag}(\Sigma)^{-\frac{1}{2}} \tag{5.5}$$

where diag(Σ) refers to the matrix formed by taking only the diagonal elements of Σ. The correlation matrix for our example exercise is illustrated in Fig. 5.3. We encourage the reader to compare the values of this matrix with distributions in Fig. 5.2.

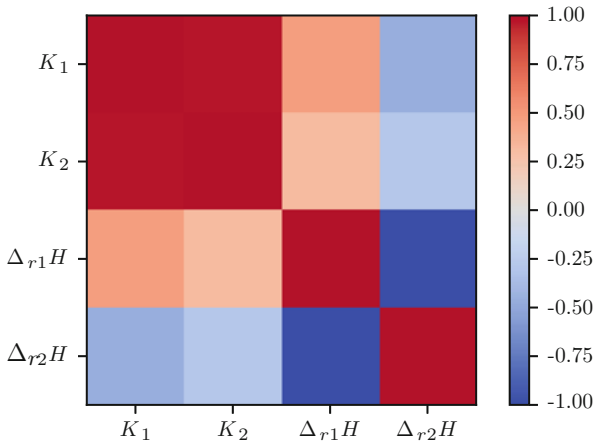

Fig. 5.3 Correlation matrix for the ensemble of best fit parameters in Figs. 5.1 and 5.2

To summarize our discussion to this point, we first find our "best fit" estimates of the true parameters by minimizing the chi-squared cost function. Next, we would like to know something about the distribution of other best fit estimates we could have expected if we had repeated this process many times. In practice, of course, we will not repeat our experiment hundreds of times in order to generate the distributions in Figs. 5.1 and 5.2. Instead, the goal of statistics is to estimate this distribution from the data available after one experiment. The idea motivating much of statistics is that although the distributions in Figs. 5.1 and 5.2 are inaccessible, we can generate similar distributions using the best fit values as surrogates for the true parameter values. The distribution of fits around the best fit parameters will be similar to those we would have found around the true values. In this way we can estimate the covariance and correlation for our parameter estimates.

5.7 Asymptotic Inference, Jackknife, and Bootstrap

To a first approximation, the distribution of points in Fig. 5.2 is nearly Gaussian. Mathematically, this is because the random numbers we added to make the observations were small enough that the best fits were close to the true parameter values. It can be shown under fairly reasonable assumptions that when observed data are sufficiently informative so as to constrain the best fits to a fairly small interval, the distribution of best fits is well-approximated by a multivariate Gaussian distribution. This is known as the asymptotic limit. When this approximation holds, everything about the distribution of best fits can be summarized in the covariance matrix, Σ, as we saw in Sect. 5.6.

In the asymptotic limit, the (inverse) covariance matrix is particularly easy to find. It is given by

$$\left(\Sigma^{-1}\right)_{\mu\nu} = \frac{M-N}{\chi^2\left(\hat{\theta}\right)} \sum_{i=1}^{M} \frac{\partial y(t_i;\theta)}{\partial \theta_\mu} \frac{\partial y(t_i;\theta)}{\partial \theta_\nu}, \tag{5.5}$$

where the derivatives are evaluated at the best fit, $\hat{\theta}$.

Notice that the only thing necessary to estimate the covariance in the asymptotic limit is a knowledge of (1) the size of the model errors at the best fit and (2) the derivatives of the residuals with respect to each of the parameters. The derivatives of the model with respect to parameters can be estimated by finite differences. Usually these derivatives are calculated as part of the fitting algorithm, in which case the covariance matrix can be obtained at the same time as the best fit.

When more rigorous confidence intervals are required that go beyond simple asymptotic inference, we suggest two approaches: jackknife resampling and bootstrapping. These methods have the advantage that they can be performed using the same machinery that generated the best fit. In jackknife resampling, for concreteness we assume the model fits M observations. The jackknife procedure then considers M separate fitting problems. In each instance, one of the observations is left out of the data set, and the least squares estimate is found for the remaining $M - 1$ observations. In this way, we generate M estimates. From this ensemble of "best fits," the formulas in Eqs. 5.3, 5.4, and 5.5 can be used to estimate the confidence intervals.

Bootstrapping is similar to jackknife in that it finds best fits to combinations of the available observations. The bootstrap method consists of fitting the model to M observations drawn randomly from the original data set with replacement. The caveat that we select randomly with replacement is necessary so that we do not get back the same set of observations each time. Typically about $1/e = 37\%$ of the observations will be duplicates. A large number of best fits can be found using these artificial data sets, and the covariance among parameters can be estimated from this ensemble as in Eqs. 5.3, 5.4, and 5.5.

Both the jackknife and bootstrap methods are straightforward to implement using the same computational machinery that generated the best fit. In contrast, more sophisticated statistical tools, such as Markov Chain Monte Carlo (MCMC) sampling (for the case of Bayesian inference) and parameter profiles (for Frequentist methods), require different theoretical and computational tools and are beyond the scope of this brief.

5.8 Global Fits to Multiple Data Sets

In many cases, data are available from multiple experiments done under different experimental conditions (e.g., differing concentrations) for the same set of chemical reactions. In this case, parameters can be estimated by simultaneously fitting all of

the available data. If $\chi_j^2(\theta)$ is the chi-squared cost function for the j-th experiment, then the global best fit can be found by minimizing

$$\chi^2(\theta) = \sum_j \chi_j^2(\theta). \tag{5.6}$$

Naively summing the cost for each experiment will likely be sufficient in many cases; however, there are some potential problems to be aware of. In particular, if the scale of the data varies significantly between experiments, it may be necessary to apply more sophisticated methods. For example, if the observed heat per injection for one experiment is ten times bigger than that for another, then the final cost will be dominated by the errors in the first experiment, and the information from the second experiment will have little bearing on the best fit. The solution is to weight the contribution to the cost from each experiment differently.

$$\chi^2(\theta) = \sum_j \omega_j \chi_j^2(\theta). \tag{5.7}$$

In Eq. 5.7, we have introduced weights ω_j, not be confused with the weights, w, in Eq. 5.1. The weights should be chosen so that the errors in each experiment are on the same scale. These weights could in principle be selected automatically (e.g., as part of a maximum likelihood estimate), but the details of how to accomplish that are beyond the scope of this brief.

In general, it is best practice to simultaneously fit all available data when possible. Global fits, by incorporating all experimental data, produce more accurate parameter estimates and smaller statistical uncertainties. Global fits can also help reveal problems in the model, when the best fit fails to give a good fit to all data simultaneously.

Chapter 6
Related Topics in Calorimetry

6.1 Analytical Applications of Titration Calorimetry

Many analytical applications of titration calorimetry were developed beginning in the 1960s when thermistors became available. Thermistors, with time constants <1 s and sufficient sensitivity to resolve a few micro-degrees, provided a convenient way to make rapid measurements of temperature that made continuous titration and single injection methods of analysis feasible with very simple equipment. These methods, variously known as thermometric titration, enthalpy titration, enthalpic injection, calorimetric titration, etc., were the forerunners to what eventually became known as isothermal titration calorimetry (ITC). In the titration methods, endpoints and thence concentrations of reactants are indicated by a change in heat production. In the injection methods, the measured amount of heat is divided by the $\Delta_r H$ value to determine the amount of analyte. Several articles and reviews on analytical applications of titration calorimetry are given in the bibliography.

6.2 Temperature Scanning Calorimetry

As mentioned earlier, reactions can be initiated by a change in temperature, and this is implemented in temperature scanning calorimetry, aka differential scanning calorimetry or DSC. This method has been applied most commonly to determine thermodynamics, i.e., $\Delta_r H$ and $\Delta_r S$, of phase changes. A secondary use has been determination of heat capacities of materials. Determination of equilibrium constants for complexes with large K and large exothermic $\Delta_r H$ values at higher temperatures is also possible with DSC (Dukhopelnikov et al. 2013 and refs therein).

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https://doi.org/10.1007/978-3-319-78250-8_6

6.3 Pressure Scanning Calorimetry

Pressure scanning calorimetry has been used primarily to determine the compressibility of materials and thence the effect of pressure on thermodynamic and kinetic parameters (Randzio 2003, 2007). A method called pressure perturbation calorimetry (PPC) has been used to obtain the partial molar cubic expansion coefficients and relative partial molar volumes of macromolecules as a function of temperature (Kujawa and Winnik 2001). These parameters can be related to the hydration state of the macromolecule.

6.4 Biocalorimetry

The earliest reported use of quantitative calorimetry was to determine metabolic heat rates which were used to prove that respiration was a slow combustion (Lavoisier and LaPlace 1780). While not technically within the scope of "titration calorimetry," the use of heat conduction and power compensation calorimetry to measure metabolic rates is mentioned here for completeness. Biocalorimetry falls within the scope of measuring rates of reactions and is currently a common application of calorimetry (Wadsö and Hansen 2015). Much of the discussion of chemical reaction kinetics also applies to measurement of metabolic rates.

Chapter 7
Self-test Questions

After studying Chaps. 1 through 5, try your hand at answering the following questions to see if you remember and understand the major concepts. The answers are given in Chap. 8, but do not peek until you have given it an honest try.

1. A calorimeter has a time constant of 15 s. What is the response time?
2. The data in Fig. 7.1 were obtained by titration of a solution containing a mixture of an amine and a sodium salt of a carboxylic acid with a strong acid. Calculate the enthalpy changes for the reactions and the heat of dilution of the titrant. How do you know which is the amine reaction and which is the carboxylate reaction? What is the ratio of moles of amine to carboxylate ions?
3. A protein has multiple binding sites for an unreactive small molecule. The titration curve is given in Fig. 7.2. What can be said about the stoichiometry, binding constants, and enthalpy changes for binding?
4. Injection of 0.15 mL of 0.05 M sulfamic acid in 0.1 M HCl into 2.5 mL of a nitrite solution in 0.1 M HCl gave a temperature rise of 0.0053 °C in a calorimeter with a calorimetric constant of 11.21 J/°C. The reaction is

$$\begin{aligned} NH_2SO_3H(aq) + HNO_2(aq) &= N_2(g) + HSO_4^-(aq) + H_2O(l) + H^+(aq) \quad \Delta_r H \\ &= -402 \text{ kJ/mole.} \end{aligned}$$

   Calculate the concentration of nitrite in the solution.

5. Titration of a ligand into a protein solution in two different pH 7.00 buffers, bicarbonate and phosphate, gave $\Delta_r H$ values of −12.1 and −6.6 kJ/mole, respectively. How many protons were released by ligand binding? The $\Delta_r H$ for ionization of $H_2PO_4^-$ is +3.6 kJ/mole and for H_2CO_3 is +9.15 kJ/mole.
6. The data in Fig. 7.3 were obtained with a titration of a solution of an aromatic amine in hexane into a suspension of 0.5 g of zeolite in hexane. Calculate the maximum capacity of the zeolite to absorb the amine in moles of amine/g of zeolite and the heat of absorption in kJ/mole.

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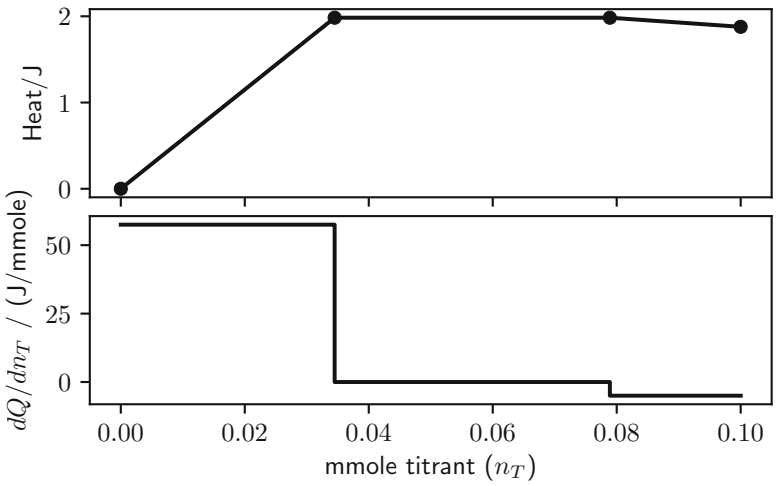

Fig. 7.1 Titration of a mixture of an amine and a sodium carboxylate with strong acid

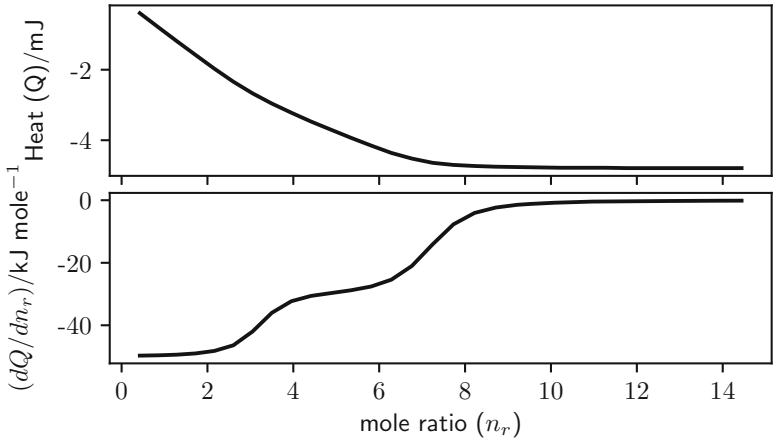

Fig. 7.2 Titration curves for titration of a small molecule into a protein with multiple binding sites

7. Calculation of K and $\Delta_r H$ for a 1:1 reaction from the curves in Fig. 7.4 gave $n = 1.00$, $\Delta_r H = -0.02$ kJ/mole, and $K = 1.87 \times 10^{15}$. Is this a believable answer? What is wrong?
8. Some typical volumes and detection limits of calorimeters are given in the following tables along with some equilibrium constants and $\Delta_r H$ values.

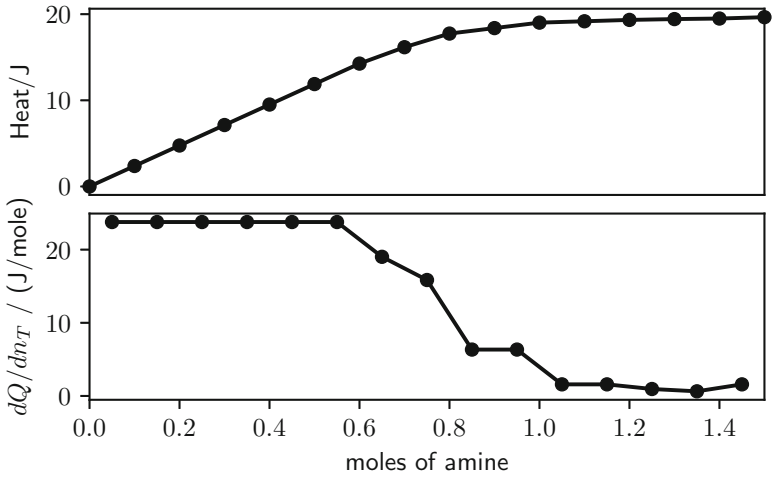

Fig. 7.3 Absorption of dissolved molecules on a solid sorbent is modeled with adsorption isotherms. The most common isotherm is the Langmuir adsorption isotherm, $\vartheta = K[B]/(1 + K[B])$, where ϑ is the fraction of sites occupied by B on the absorbent. Some systems require a more complex model such as the Freundlich adsorption isotherm: $a/m = K[B]^{1/s}$ where a is the quantity of adsorbate adsorbed in moles, m is the mass of the adsorbent, $[B]$ is the concentration of adsorbate, and K and s are empirical constants for each adsorbent-adsorbate pair at a given temperature

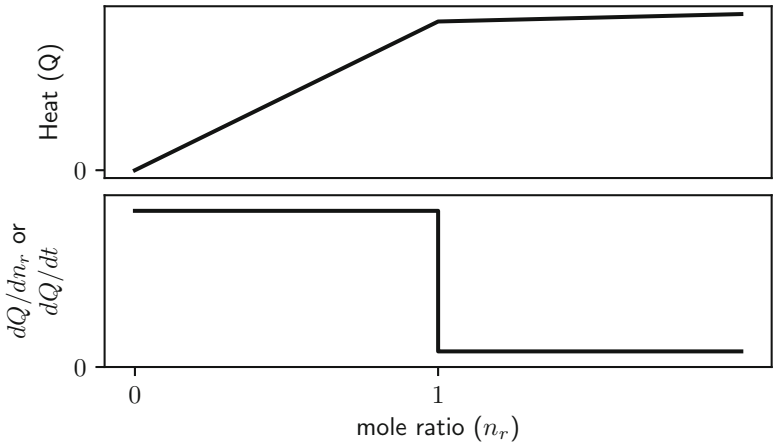

Fig. 7.4 Titration data for a quantitative 1:1 reaction

Calorimeter	Volume/mL	Detection limit/δQ	V/δQ in mL/μJ
Power compensation #1	0.3	0.1 μJ	3
Power compensation #2	1	0.2 μJ	5
Heat conduction	10	0.1 mJ	0.1
Temperature change #1	25	0.5 mJ	0.05
Temperature change #2	100	1 mJ	0.1

(continued)

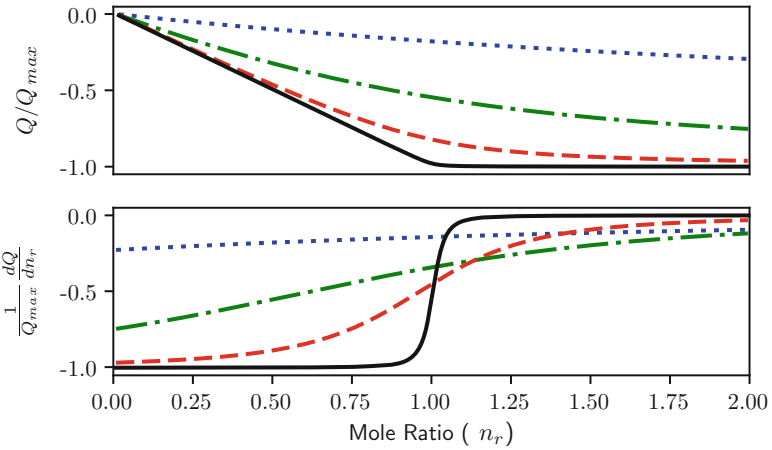

Fig. 7.5 Titration curves obtained with differing total concentrations of reactant and titrant

K of 1:1/nmole mL^{-1}	$\Delta_r H$/μJ nmol^{-1}	$K/\Delta_r H$/mL μJ^{-1}	Suitable calorimeters
0.1	30	0.003	None
1	30	0.03	Temperature change #1
10	30	0.3	Heat conduction and temperature change #1 and #2
100	30	3	Power compensation #1 and #2
1000	30	30	None

Use the $V/\delta Q$ data on the calorimeters in the first table and Fig. 3.9 to determine the range of suitable $K/\Delta_r H$ values for each calorimeter. Do you agree with the answers given in the last column in the second table?

9. Titration of a solution of *A* into a solution of *B* gave the curves shown in Fig. 7.5 for a range of concentrations. Calculation of K and $\Delta_r H$ from these data assuming a 1:1 reaction gave very different values for K at different concentrations. What is the most likely cause?
10. The table below shows two sets of data at 25 °C for a titration of *A* into *B* to give *AB*.

	First set			Second set		
Point #	[*A*] total/M	*Q*/J	*dQ/dn*, kJ/mole	[*A*] total/M	*Q*/J	*dQ/dn*, kJ/mole
0	0	0		0	0	
1	0.002015	1.892	9.390	0.005081	5.280	10.392
2	0.004031	3.686	8.899	0.010162	9.976	9.242
3	0.006046	5.413	8.571	0.015243	14.178	8.270
4	0.008062	7.002	7.882	0.020323	17.898	7.323
5	0.010077	8.518	7.524	0.025404	21.143	6.387

(continued)

	First set			Second set		
Point #	[*A*] total/M	*Q*/J	*dQ/dn*, kJ/mole	[*A*] total/M	*Q*/J	*dQ/dn*, kJ/mole
6	0.012093	9.952	7.113	0.030485	24.632	6.867
7	0.014108	11.279	6.586	0.035566	26.585	3.844
8	0.016123	12.513	6.124	0.040647	28.821	4.401
9	0.018139	13.696	5.868	0.045728	30.849	3.991
10	0.020154	14.797	5.464	0.050809	32.654	3.552
11	0.022170	15.831	5.129			

Which data set is better for determining K and $\Delta_r H$? Why?

Fitting the data gave $K = 100\ \mathrm{M}^{-1}$ and $\Delta_r H^0 = -22$ kJ/mole for both sets of data. Are these values reasonable? Calculate the entropy change, $\Delta_r S$, for the reaction. Remember that $\Delta_r G^0 = -RT\ln K = \Delta_r H^0 - T\Delta_r S^0$.

11. Use Eq. 3.24, $KC = \alpha/(1-\alpha)^2$, and a spreadsheet to calculate values of α at the equivalence point for $KC = 1$, 10, 50, 100, 200, 300, 400, 500, 1000, 5000, and 10,000. How do your results explain why the calorimetric method for determination of equilibrium constants works best when $50 < KC < 500$?
12. What is the maximum time constant of a calorimeter that could be used to determine the kinetics of a first-order reaction with a rate constant of $0.005\ \mathrm{s}^{-1}$?
13. A titration curve is run with $KC = 5$. Is there likely to be a clearly discernable endpoint? If not, is it possible to obtain a value for n without use of any other data?

Chapter 8
Self-test Key

1. 90 s. Time constant (15 s) × six = response time. Refer to Chap. 1.
2. The enthalpy change, $\Delta_r H$, for the first reaction is (2.0 J/0.038 mmole) = −52 J/mmole or −52 kJ/mole. $\Delta_r H$ for the second reaction is 0 kJ/mole. $\Delta_r H$ for dilution of the titrant is (−0.1 J/0.021 mmole) = +4.8 kJ/mole. Amines are usually more basic than carboxylates, so the expectation is that the amine is the first reaction, but the proof is the $\Delta_r H$ value, −50 kJ/mole is a defining characteristic for protonation of amines, and 0 kJ/mole is a defining characteristic of protonation of carboxylate groups. The mole ratio of amine to carboxylate is (0.038/0.041) = 0.93. Refer to Sect. 3.9.
3. The stoichiometry is three stronger binding sites and four weaker binding sites. Both kinds of sites are cooperative, i.e., $K1 \approx K2 \approx K3 < K4 \approx K5 \approx K6 \approx K7$. The enthalpy change, $\Delta_r H$, for the strong binding site is −50 kJ/mole and for the weaker binding site, −30 kJ/mole. The rounded endpoints show that the binding constants β_3 and β_{4-7} could be calculated from these data. Refer to Chaps. 1 and 5 and Sects. 3.4 and 3.8.
4. The concentration of nitrite is 59 μM. (11.21 J/°C)(0.0053 °C) = 0.0594 J; (0.0594 J)/(−402,000 J/mol)) = 0.1478 μmols product; 1:1 relationship, so the concentration is (0.1478)/(2.5 mL) = 59 μM. There is a large excess of sulfamic acid, i.e., (0.05 M)(10^6 μmole/mole)(10^{-3} L/mL)(0.15 mL) = 7.5 μmoles. The temperature measurement limits the answer to two significant digits. Refer to Sect. 3.3.
5. One H^+ released. The reactions are

$$L + P + x\mathrm{HPO}_4^{2-} = LP + x\mathrm{H_2PO}_4^- \qquad \Delta_r H = -6.6\ \mathrm{kJ/mol} \qquad (8.1)$$

$$L + P + x\mathrm{HCO}_3^- = LP + x\mathrm{H_2CO_3} \qquad \Delta_r H = -12.1\ \mathrm{kJ/mol.} \qquad (8.2)$$

Subtract the second reaction from the first to get

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$$x\mathrm{HPO}_4^{2-} + x\mathrm{H_2CO_3} = x\mathrm{H_2PO_4^-} + x\mathrm{HCO_3^-} \qquad \Delta_r H = -6.6 + 12.1,$$
$$= +5.5\ \mathrm{kJ/mol}, \tag{8.3}$$
$$\mathrm{HPO}_4^{2-} + \mathrm{H_2CO_3} = \mathrm{H_2PO_4^-} + \mathrm{HCO_3^-} \qquad \Delta_r H = -3.6 + 9.15$$
$$= +5.5\ \mathrm{kJ/mol}. \tag{8.4}$$

Therefore, $x = 1$.

Another method is to determine the slope of a plot of the measured $\Delta_r H$ values on the y-axis and the enthalpy changes for protonation of the buffers ($\Delta_{BH}H$) on the x-axis. The slope is the number of protons. The slope is positive if protons are released from the active site and negative if protons are taken up by active site. Refer to Baker and Murphy (1996).

6. The plot shows an endpoint at ≈0.8 moles of amine. A better value could be obtained from the intersection of two line extrapolated from the linear portions of the plot before and after the endpoint. So the capacity is 0.8 mole/0.5 g = 1.6 moles per gram. The heat of absorption is ≈20 J/0.8 mole = 25 J/mole of amine. Refer to Sects. 3.2 and 3.3.
7. The values of K and $\Delta_r H$ found from fitting the curve are not reasonable. Assuming that concentration data were entered correctly, the sharp endpoint shows the value of KC is too large to apply this method, so the equations become indeterminate and the fitting procedure finds a product of K and $\Delta_r H$ that is at a false minimum or limit. Most fitting procedures will always find an answer, even if it is incorrect. Also, the enthalpy change found from fitting the data does not agree with the enthalpy change estimated directly from the curve. To measure a K this large, C would have to be at picomolar to femtomolar concentrations, and at such low concentrations, the heat from the reaction would be below the detection limit of the calorimeter. Another possible reason is that the concentration data are incorrect by orders of magnitude, e.g., entered as moles/L instead of μmoles/L. Refer to Sects. 3.5, 3.10, and Chap. 5.
8. Find the $V/\delta Q$ value for a calorimeter on the x-axis in Fig. 3.9, and then find the range of suitable $K/\Delta_r H$ values between the lines on the plot. If the $K/\Delta_r H$ value falls in the suitable range, the calorimeter is applicable to determine K by the calorimetric method. Refer to Sect. 3.5.
9. If K and $\Delta_r H$ are not constant over a range of concentrations, it shows that the model being used to fit the data is incorrect. Since the endpoint clearly shows the reaction stoichiometry is 1:1, the reaction product must be a polymer of AB, i.e., A_2B_2 or A_3B_3, etc. Refer to Sect. 3.8.
10. Plots of the data look like this (Fig. 8.1):

The first set is at a lower concentration and reached a lower ratio of titrant to reactant. The second set reached a higher ratio but has two points that are off the line. Both sets would likely give about the same results. Refer to Chap. 5. The results are reasonable for three reasons, the shape of the curve agrees with a quite small value of K, and the value of $\Delta_r H$ is reasonable, being approximately twice the value of dQ/dn

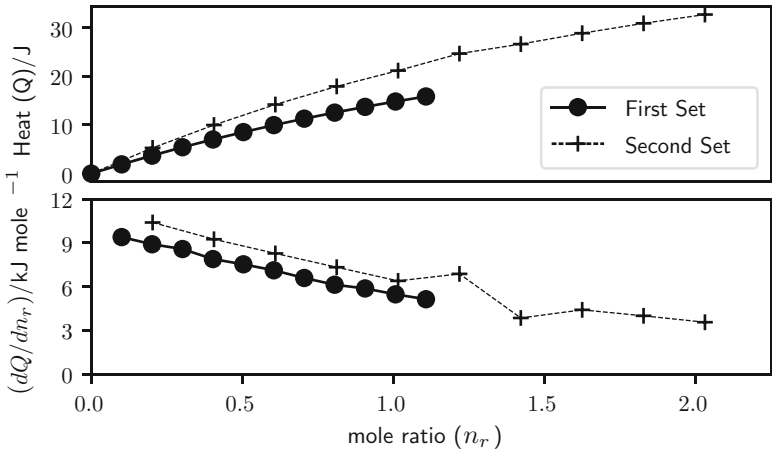

Fig. 8.1 Plot of total heat and incremental heat per mole against mole ratio for an incomplete reaction

for the first injection of titrant which is in agreement with a very small K value, and the agreement at different concentrations shows the model is likely correct. Refer to Sects. 3.4 and 3.5.

$$\Delta_r G^\circ = -RT\ln K = -\left(8.314\ \text{J K}^{-1}\text{mole}^{-1}\right)(298\ \text{K})(4.605)$$
$$= -11410\ \text{J mol}^{-1} \tag{8.5}$$

$$\Delta_r S^\circ = \frac{\Delta_r H^\circ - \Delta_r G^\circ}{T} = \frac{-22000 + 11410}{298} = -35.5\ \text{J mol}^{-1}\text{K}^{-1} \tag{8.6}$$

Refer to Chap. 2.

11. Rearrange the equation to $0 = KC\alpha^2 - (1 + 2KC)\alpha + KC$, and use the quadratic equation to solve for α. ($\alpha = [(1 + 2KC) \pm ((1 + 2KC)^2 – 4(KC)^2)^{0.5}]/2KC$)

KC	1	10	50	100	200	300	400	500	1000	5000	10,000
α	0.38	0.72	0.86	0.90	0.93	0.94	0.95	0.95	0.96	0.98	0.99

The reaction is too incomplete at values of $KC < 50$ and too close to completion at values of $KC > 500$ to give the best results. But note these KC limits are "fuzzy," not exact. Refer to Sect. 3.5.

12. The rate constant for a first-order rate law is the reciprocal of the time constant, so $\tau_{rxn} = 1/k_{rxn} = 1/0.005\ \text{s}^{-1} = 200$ s is the time constant of the reaction. The maximum time constant of the calorimeter must be at least five times less than this, so $\tau_{cal} < 40$ s. Refer to Sect. 4.1.
13. No, and n cannot be evaluated from the data by fitting the curve. Refer to Sect. 3.8 and Chap. 5.

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