Netsu Sokutei **28** (2) 63-67

解　説

Standards in Titration Microcalorimetry

Ingemar Wadsö

(Received November 28, 2000; Accepted December 5, 2000)

The importance of uniform terminology and standardised chemical calibration and test processes for titration microcalorimetry are discussed. Calorimeters are normally calibrated electrically, but in the case of microcalorimeters, results from such calibration experiments can easily lead to significant systematic errors. The quality of results will often improve if a chemical calibration technique is used. In order to test the properties of a calorimeter and to validate the results, it is important to have available suitable test reactions. References are given to some standardised chemical calibration and test processes for titration microcalorimetry.

1. Introduction

In all areas of science it is essential that a well-defined and generally understood terminology is used. Scientific measurements should be conducted with carefully calibrated instruments and results should be validated by suitable standardised test processes. In the present brief report needs for a uniform terminology and standardised chemical calibration and test processes in the field of titration microcalorimetry are discussed.

The term "microcalorimeter" is commonly used for calorimeters designed for use in the microwatt range. "Isothermal microcalorimeters" are normally not used under strict isothermal conditions. However, for the small heat quantities evolved or absorbed in microcalorimetric experiments, it is normally possible to treat the results as if the measurement was conducted isothermally. "Nanocalorimeters", usually indicating a detection limit approaching a few nanowatt, are in this paper not distinguished from "microcalorimeters".

A more general discussion of needs for standards in isothermal microcalorimetry has earlier been reported.[1] IUPAC guidelines on these matters are presently under review.[2]

2. Nomenclature and units

Where possible, nomenclature and symbols should follow the guidelines accepted by IUPAC.[3] Results should be expressed in terms of SI units.[3] In some specific cases no applicable recommendation exist in which cases it is important that terms "invented" by individual scientists are not in conflict with the general framework used by the unions. The use of showy advertising terms of the type "ultra-sensitive" carries little information in the description of a microcalorimeter and should not be encouraged.

A few important terms used in describing a calorimeter will be briefly discussed. The calorimetric principle (see below) employed should always be reported. The time constant should be reported, especially for heat conduction microcalorimeters. The "repeatability" is a measure of the scatter between the results of a series of repeated measurements. "Repeatability" can be expressed in terms of the standard deviation of the mean.[4-6] The "uncertainty" is a quantity referring to the difference between a determined (mean) value and the true value for the measured quantity. "Repeatability" and "uncertainty" are identical to "precision" and "accuracy", respectively. These latter terms are not any more recommended.[4]

A value for the "uncertainty" is usually obtained by combining a value for "repeatability" and an estimated systematic error in the measurement. However, it is frequently very difficult to make realistic estimates of systematic errors in result from microcalorimetric experiments. It is therefore useful to base such estimates

on the result from a standard test process, of a similar type as the studied process.

The term "limit of detection" means the smallest heat quantity or thermal power (=heat production rate), which can be determined with an acceptable uncertainty, under specified experimental conditions. The term "sensitivity" is often used for the property "limit of detection", a practice which is discouraged by IUPAC.[4-6] The sensitivity (S) of an instrument is defined as the slope of a calibration curve. In case of a calorimeter this means that S is equal to the inverse of the calibration constant. For example, for a thermopile heat conduction microcalorimeter S is expressed in units of watt per volt (W V^{-1}).

Numerical data should be given in SI units.[3] Thus, "calorie" should never be used - the internationally accepted unit for energy is "joule", symbol J.

3. Calorimetric principles and calibration constants

Calorimetric signals are standardised by the release of an accurately known heat quantity, Q, or thermal power, $P = dQ/dt$. The result of a calibration experiment is normally expressed in terms of a calibration constant, ε. As we will see from the next few paragraphs, the physical meaning of ε depends on the calorimetric principle employed. The most important calorimetric principles used in isothermal microcalorimetry are: the adiabatic, the (thermopile) heat conduction and the power compensation principle. Normally, isothermal microcalorimeters are designed as twin instruments and differential signals are measured.

3.1 Adiabatic instruments

In an adiabatic calorimeter no heat exchange takes place between the calorimetric vessel and the surroundings. For an exothermic process the temperature of the reaction vessel will thus increase and following an endothermic processes the temperature will decrease. In a strict sense an adiabatic calorimeter can thus never be used isothermally. Calorimeters used for measurements of short processes are often semi-adiabatic ("isoperibol"). Results obtained with such instruments should in accurate work be corrected for the heat exchange with the surroundings.[7,8]

The heat quantity evolved or absorbed in an experiment with an adiabatic calorimeter is in the ideal case equal to the product of the temperature change, ΔT, and the heat capacity of the vessel, including its content, C.

$$Q = C\ \Delta T \tag{1}$$

The calibration constant, ε_a, is equal to the quotient between the heat quantity and the temperature change and is thus identical with the heat capacity for the vessel and its content (in the ideal case).

$$Q = \varepsilon_a\ \Delta T \tag{2}$$

$$P = \varepsilon_a\ dT/dt \tag{3}$$

A change in the heat capacity of the content of the calorimetric vessel, for example following an injection in a titration experiment, will thus change the calibration constant.

3.2 Thermopile heat conduction calorimeters

Most isothermal microcalorimeters in current use are of the thermopile heat conduction type. In such instruments heat is exchanged between the reaction vessel and a surrounding heat-sink (normally a metal block) for which the temperature is essentially constant. The heat transfer takes place through a thermopile wall and is thus recorded as a potential signal. In a well-stirred microcalorimetric titration vessel there are no significant thermal gradients and the Tian equation will hold:

$$P = \varepsilon_c\ [U + \tau\ (dU/dt)] \tag{4}$$

where ε_c is the calibration constant, U the measured thermopile potential, and τ the time constant. The time constant is in the ideal case defined by

$$\tau = C/G \tag{5}$$

where C is the heat capacity of the reaction vessel with its content and half of the thermopile wall. G is the heat conductance of the thermopile. A more complex expression should be used in cases where temperature gradients in the reaction vessel are significant.

In the ideal case ε_c is equal to the ratio between G and the Seebeck coefficient for the thermocouple material. Thus, in contrast to the case for adiabatic calorimeters, the calibration constant for a thermopile heat conduction calorimeter is in the ideal case independent of the heat capacity of the system. In a practical titration experiment ε_c will normally not change significantly when a sample is injected into the vessel.

Under steady state conditions, eq.(4) will be simplified to

$$P = \varepsilon_c \, U \qquad (6)$$

The heat released in the calorimetric vessel is obtained by integration of eqs.(4) or (6). If the initial and final potentials are the same (usually the baseline value for which $U = 0$), the simple expression (7) is obtained.

$$Q = \varepsilon_c \int U dt \qquad (7)$$

The time constant for a titration microcalorimeter is typically in the order of one or a few minutes, which is much longer then for adiabatic and power compensation calorimeters. However, by "dynamic correction" techniques it is possible to shorten the experimental time for a stepwise titration experiment to the same level as for the other principles, without any loss of accuracy.[9)]

3.3 Power compensation calorimeters

In a power compensation calorimeter the thermal power from an exothermic process is balanced by a cooling power, in microcalorimetry normally by use of Peltier effect cooling. For an endothermic process corresponding compensation is made by the release of electrical energy in an electrical heater (*e.g.* the calibration heater) or simply by reversing the Peltier effect current.

In a special type of power compensation micro-calorimeter,[10)] which currently frequently is used in titration experiments, the temperature is allowed to increase at a constant rate, as in a DSC but very slowly. The thermal power released in the reaction vessel is in that case compensated for by a change of the electrical heating power.

4. Calibration and test processes

Most titration microcalorimeters are calibrated by the release of electrical power in an electrical heater positioned in the reaction vessel or in its close proximity. Electrical calibration techniques are precise and convenient and the measurement of electrical power or energy can easily be made with a lower uncertainty than needed in any practical calorimetric experiment. However, the thermal power from an electrical calibration heater may give rise to a significantly different temperature distribution or heat flow pattern, compared to an identical thermal power released during an investigated process. The calorimetric sensor (a thermometer or a thermopile used as a heat flow sensor) can therefore respond differently for the same heat quantity or thermal power released in the electrical calibration experiment and in the practical calorimetric measurement. Systematic errors from such effects are influenced by the calorimetric principle employed, design of vessel and calibration heater, stirring efficiency etc. For a typical titration microcalorimeter such systematic errors can easily amount to about 5 %. It is therefore felt that the quality of results in titration microcalorimetry would improve if some instruments were calibrated by use of chemical calibration processes. In any case, standardised chemical test processes should be used much more frequently than at present, in all kinds of microcalorimetric experiments. Such processes are also invaluable in the training of users of the instruments.

Any process that can be conducted in a calorimeter under standardised conditions and has sufficiently well known thermochemical properties, can be used as a test or calibration process. Reaction systems recommended as reference materials in physical chemistry, including calorimetry, were reported in a IUPAC monograph edited by Marsh.[11)] More recently a compilation of reference materials for calorimetry and thermal analysis, prepared under the auspices of ICTAC, was edited by Sabbah.[12)] Several chemical systems have been specifically proposed for use in isothermal microcalorimetry, in particular with respect to aqueous solutions and in the study of living cellular systems.[1,14)] In the next section a few chemical processes are discussed with reference to their use as test and calibration processes in titration microcalorimetry. In the next few paragraphs some chemical calibration and test processes useful in titration microcalorimetry will be discussed.

4.1 Aqueous dissolution and dilution of propan-1-ol in water

Propan-1-ol is readily available in sufficiently pure form and the enthalpies of dissolution and dilution are well known for the temperature range 283～348 K.[14)] Further, propan-1-ol is inexpensive, stable and non-corrosive.

The dissolution of propan-1-ol has proved to be a reliable and convenient calibration and test process in isothermal microcalorimetry, in particular in connection with experiments where small volumes of liquid are

injected into a much larger volume of solvent or solution. Eq.(8)[13] summarises values for the enthalpies of solution of propan-1-ol in water, to form infinitely dilute solutions, $\Delta_{sol}H_m^{\infty}$. The eq.is valid for the temperature range 288～348 K. Propan-1-ol molalities＜0.017 mol kg^{-1} (mass fraction＝0.001) can normally be considered as infinitely dilute.

$$\Delta_{sol}H_m^{\infty}(T)/\text{kJ mol}^{-1} = -15.880 + 0.2450 \cdot (T-273.15) - 6.474 \times 10^{-4} (T-273.15)^2 \quad (8)$$

It should be noted that the ΔC_p for the process is high, which can be advantageous if the user wants to test the ability to determine this very important property.

At ambient temperatures the heat quantity evolved in the dissolution of propan-1-ol is sometimes too large for use in titration calorimetry. In such cases the dilution of aqueous propan-1-ol solution can be more useful.[13] Dilution enthalpies expressed as interaction coefficients for the dilute region have been reported for the temperature range 284～318 K.[14,15]

The dilution of mass fraction＝0.1000 (1.849 mol kg^{-1}) propan-1-ol solution to infinitely dilute solution will give $\Delta_{dil}H_m^{\infty}$=−(1572±44) J mol^{-1} at 298.15 K.[16] For use as a calibration and test process, results reported[13,16] can be expressed as: the enthalpy of dilution of 1 mg aqueous propan-1-ol, mass fraction 0.1000, equals 2.57±0.02 mJ, provided that the final propanol concentration is≦1.4 mg per mg of water.

4.2 Dilution of aqueous sucrose solutions

The enthalpy of dilution of aqueous sucrose solutions, is well known over a wide range of concentrations and temperatures,[11-13,17] and has been used extensively for tests of microcalorimeters where two liquids are mixed. Concentrated sucrose solutions, mass fraction 0.15～0.25, are quite viscous and can be difficult to mix with water. This dilution process is therefore particularly useful in tests of mixing efficiency. $\Delta_{dil}H$ values are summarised by eqs.(9)-(11)[13]

$$\Delta_{dil}H = A(m_2 - m_1) - B(m_2^2 - m_1^2) \quad (9)$$

where m_1 and m_2 are the initial and final molalities, respectively, of the sucrose solution.[13] (symbols for molalities were[13] erroneously given in the reverse order).

$$A(T)/\text{J kg mol}^{-2} = -834.9 + 4.719\ T \quad (10)$$

$$B(T)/\text{J kg}^2\text{ mol}^{-3} = -389 + 2.74\ T - 4.5 \times 10^{-3} T^2 \quad (11)$$

$\Delta_{dil}H$ values calculated by use of eqs.(9)-(11) are judged to be accurate to about 1 % for the ambient temperature range and for m_1 and m_2 in the ranges 0.1～2.0 mol kg^{-1} and 0.01～0.2 mol kg^{-1}, respectively.

4.3 Acid-base reactions

Aqueous acid-base reactions have frequently been used as calibration or test processes in solution calorimetry. In most cases dilute hydrochloric acid (or some other strong acid, readily available at standardised concentrations) has been reacted with a large excess of dilute sodium hydroxide solution. The neutralisation of amine buffer solutions, in particular of "tris" (trishydroxymethyl aminomethane) have also been used.

Recommended values for protonation of hydroxyl ions at 298.15 K and infinite dilute solution are[17]: ΔH^{∞}= − 55.81 kJ mol^{-1} and ΔC_p^{∞} = 224 J mol^{-1} K^{-1}. Experimental values should normally be corrected for the enthalpy of dilution of the comparatively concentrated injected solution, using experimental data.

Neutralisation processes can be convenient to use as calibration standards and in the test of microcalorimeters, but some experimental problems should be pointed out. In experiments where one reaction component is mixed with a large excess of the other component, the reaction may go to completion even if the mixing efficiency in the calorimetric vessel is very poor. Thus, a neutralisation test or calibration reaction can give a correct value even if the calorimeter, due to inferior mixing properties, will give false results in other types of measurements, for example in titration and dilution experiments.

Contamination with CO_2 can influence results significantly, in particular when a reaction is conducted in very dilute hydroxide solution. Therefore, reagents must be prepared from CO_2 free water and be protected from uptake of CO_2 during the experiments.

In many types of microcalorimetric experiments (for example titration and dilution experiments) small volumes are injected into the reaction vessel (typically a few μl into a few ml). The concentration of the injected solution may therefore be comparatively high and injection tubes made from "acid proof" stainless steel, can be corroded by an HCl solutions.

4.4 Ligand binding processes

The 1:1 binding reaction between Ba^{2+} and the macrocyclic compound 18-crown-6 (1,4,7,10,13,26-

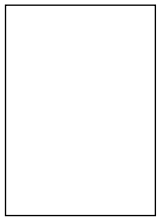
hexaoxacyclo-octadecane) in aqueous solution has proved to be a reliable and convenient test process in titration microcalorimetry.[13] The reagents are inexpensive and stable and are easily available in sufficiently pure form. A small correction should normally be applied for the enthalpy of dilution of the injected barium salt solution. No significant variation in the derived thermodynamic quantities is observed when the concentration of the crown ether in the vessel is varied ($0.001 \sim 0.01$ mol l^{-1}) or for the barium chloride in the injection syringe ($0.01 \sim 0.1$ mol l^{-1}). The values for ΔH and K_c are of a magnitude suitable for calculation of precise results. Values tentatively recommended[13] for the thermo-dynamic quantities at 298.15 K: $\Delta H = -(31.42 \pm 0.20)$ kJ mol^{-1}, $K_c = (5900 \pm 200)$ mol l^{-1} and $\Delta C_p = 126$ J K^{-1} mol^{-1} ($288 \sim 310$ K).

The binding of 2'-CMP (2-cytidine monophosphate) to bovine pancreatic ribonuclease A (RNaseA) has also been used as a test process in titration microcalorimetry. From calorimetric investigations at different values for pH, ionic strength, temperature and RNaseA concentration[10,18-20] it has been found that the derived thermodynamic properties are much dependent on these variables. It has been proposed that the standard test reaction be performed at high salt concentration because the enthalpy change is less sensitive to protein concentration under these conditions.[18] Proposed standard conditions are: pH 5.5, 0.2 M potassium acetate, 0.2 M potassium chloride and 0.175 M RNaseA. Values tentatively recommended at 298.15 K[18]: $\Delta H = -(50 \pm 3)$ kJ mol^{-1}; $K_c = (120 \times 10^3 \pm 5 \times 10^3)$ mol l^{-1}. The variation in the values reported in the literature for ΔC_p is high.

No test processes have been proposed for more complex binding models or for reactions in organic solvents.

References

1) I. Wadsö, *Thermochim. Acta* **347**, 73 (2000).
2) I. Wadsö, A. E. Beezer, R. N. Goldberg, G. Olofsson, K. Murphy, J. Rouquerol, and J. Sipowska. *In preparation.*
3) I. Mills, T. Cvitas, K. Homan, N. Kallay, and K. Kuchitsu, *Quantities, Units and Symbols in Physical Chemistry*, Blackwell, Oxford (1988).
4) V. Gold, K. L. Loening, A. D. McNaught, and P. Semhi, in *Compendium of Chemical Technology. IUPAC Recommendations*, Blackwell, Oxford (1987).
5) H. Freiser and G. N. Nancollas, *Compendium of Analytical Nomenclature, IUPAC Definite Rules*, Blackwell, Oxford, 2nd ed. (1987).
6) G. Olofsson, S. Angus, G. T. Armstrong, and A. N. Kornilov, *J. Chem. Thermodyn.* **13**, 603 (1981).
7) J. M. Sturtevant, in *Physical Methods of Organic Chemistry, Vol.1, Part V*, A. Weissberger, B. W. Rossiter (Eds.), pp.347-425, Wiley, New York (1971).
8) E. H. P. Cordfunke and W. Ouweltjes, in *Solution Calorimetry*, (*Experimental Thermodynamics, Vol. IV, IUPAC Chemical Data Series, No.39*), K. N. Marsh, P. A. G. O'Hare (Eds.), pp.25-42, Blackwell, Oxford (1991).
9) P. Bäckman, M. Bastos, D. Hallén, P. Lönnbro, and I. Wadsö, *J. Biochem. Biophys. Methods* **28**, 85 (1994).
10) M. Straume and E. Freire, *Anal. Biochem.* **203**, 259-268 (1992).
11) K. N. Marsh, in *Recommended Reference Materials for the Realization of Physicochemical Properties. Sect. 9, Enthalpy*, pp.219-319, Blackwell, Oxford (1987).
12) R. Sabbah (Ed.) *Reference Materials for Calorimetry and Thermal Analysis in Thermochim. Acta* (Special Issue) **331**, 93 (1999).
13) L.-E. Briggner and I. Wadsö, *J. Biochem. Biophys. Meth.* **22**, 101 (1991).
14) D. Hallén, S.-O. Nilsson, W. Rothschild, and I.Wadsö, *J. Chem. Thermodyn.* **18**, 429 (1986).
15) F. Franks, M. Pedley, and D. Reid, *J. Chem. Soc., Faraday Trans.1* **72**, 359 (1976).
16) G. Olofsson, D. Berling, N. Markova, and M. Molund, *Thermochim. Acta* **347**, 31 (2000).
17) L. G. Hepler, E. M. Woolley, in *Water, A Comprehensive Treatise, 359 (1976) Vol.3*, p.153, F. Franks (Ed.), Plenum Press, New York (1973).
18) M. Straume and E. Freire, *Anal. Biochem.* **203**, 259 (1992).
19) M. Flogel, A. Albert, and R. L. Biltonen, *Biochemistry* **14**, 2616 (1975).
20) M. El Harrous and A. Parody-Morreale, *Anal. Biochem.* **254**, 96 (1997).



Ingemar Wadsö
Chemical Center, Physical Chemistry 1, Lund University, P.O.Box 124, S-221 00 Lund, Sweden, TEL. +46-46-222-8180, FAX. +46-46-222-4533, e-mail: ingemar.wadso@termk.lu.se