

Needs for standards in isothermal microcalorimetry

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Abstract

Calorimeters are normally calibrated electrically. In the case of isothermal microcalorimeters the heat flow pattern in electrical calibration experiments are in some cases significantly different from that of investigated processes which may lead to important systematic errors. In such cases use of chemical calibration methods should be considered. It is of general importance to conduct tests of the performance of calorimeters using well established chemical test processes. References are given to some chemical test and calibration processes for isothermal microcalorimetry conducted under ambient conditions. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Techniques in isothermal microcalorimetry have seen much improvement during the past two decades. Sensitivity and precision have been increased and working procedures have become more convenient. Current important application areas include, for example, ligand binding studies, measurement of dissolution and sorption processes, estimation of stabilities for compounds and products of technical importance and monitoring of processes of living cellular material [1].

The term ‘isothermal microcalorimeter’ is commonly used for calorimeters designed for experiments conducted in the microwatt range under essentially isothermal conditions. ‘Nanocalorimeters’, usually implying a detection limit approaching a few nano-

watt, in these papers are included in the group of ‘microcalorimeters’.

Practically all processes are accompanied by heat effects, which makes calorimetry useful as a general technique for thermodynamic and analytical investigations in physics, chemistry and biology. However, it will also make calorimetric methods uniquely vulnerable to systematic errors, for example due to processes like: evaporation, condensation, adsorption, corrosion, friction and unidentified chemical side reactions. Errors from the calibration process, normally overlooked for commercially produced instruments, can easily be significant.

Are results obtained with today’s isothermal microcalorimeters more accurate than those reported some 20 years ago? I do not know, but it is my impression that most users are not particularly concerned about possible systematic errors in their measurements, including the calibration experiments. In general, both the instrument manufacturers and users appear to be content with reporting statistical values for ‘precision’

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rather than combining such values with estimates of possible systematic errors leading to values for the ‘accuracy’.

The present brief discussion on the use of chemical test and calibration processes is partly based on earlier papers from this laboratory [2–4] and on a report presented and discussed at the (North American) Calorimetry Conference in Vancouver (1996). That report was also presented as a ‘feasibility study’ at the meeting of IUPAC’s Commission 1.2 in Osaka (1996) and a Working Party connected with IUPAC’s Commission 1.2 is presently preparing guidelines on the use of standardised chemical test and calibration processes in isothermal microcalorimetry.

2. Electrical calibrations

Normally all kinds of modern calorimeters are calibrated by the release of electrical power in a calibration heater. Electrical calibration techniques are convenient to use and the measurement of electrical power or energy can easily be made with a higher accuracy than required in any conceivable microcalorimetric experiment. However, the heat flow pattern generated by an electrical heater can be significantly different from that released by an investigated process, which may lead to serious systematic errors. Such errors are influenced by factors such as a non-ideal design of the vessel and the electrical heater, the position of the heater, the heat conductance of the vessel material, the content of the vessel and (for liquid contents) its agitation. For flow or perfusion vessels the problems can be very different from those in a stirred batch vessel. In particular some types flow-through vessels are very difficult to calibrate electrically [2,3].

Calibration errors tend to be more serious for microcalorimeters than for instruments where larger heat quantities or thermal powers are measured sometimes due to the fact that the design of microcalorimeters frequently is less ideal than that of ‘macrocalorimeters’. In thermopile heat conduction microcalorimeters it is not uncommon that a significant fraction of the thermal power produced in the reaction vessel will not pass through the thermopile, but will reach the heat sink (or other parts of the

surroundings) by the way of mechanical supports, electrical leads, air gaps etc. For example, the fraction of the heat flow passing through the thermopile may easily be less than 80% [5], which appears alarming for an instrument intended for accurate work. However, systematic errors due to such problems can still be kept small if the heat flow pattern in the electrical calibration experiment closely mimics that of the investigated process. Where possible, the calibration heater should be positioned in close thermal contact with the content of the reaction vessel. If this is a liquid it should be well stirred (or agitated by other means), both in the calibration experiment and in the measurement of the investigated process. For isothermal microcalorimeters of the heat conduction type the calibration constant is not dependent on the heat capacity of the reaction vessel and its content [5]. For such instruments it is often suitable to use an insertion heater [2] which is positioned in the reaction vessel solely during the calibration experiment.

3. Chemical test and calibration processes

In cases where it is not possible, or suitable, to let the heat flow pattern from a calibration heater closely match that of the investigated process it can be advantageous to use some kind of chemical calibration technique. Further, in accurate work it is always desirable to control the performance of the instrument, including the calibration value, by measurement of a test process resembling the investigated process. Such processes are also valuable in the training of users of the instruments. It is important to have many different test and calibration processes available in order to closely mimic the investigated processes.

Chemical substances and reaction systems judged to be suitable for such purposes in different types of ‘classical macrocalorimeters’ have been described in some detail in a IUPAC monograph edited by Marsh [6] and in the recent compilation of reference materials for calorimetry and thermal analysis, edited by Sabbah [7] and prepared under the auspices of ICTAC. Some of the systems discussed in [6,7] are referred to in the following discussion. Others have more specifically been proposed for the use in isothermal microcalorimetry, in particular for inves-

tigations of aqueous reaction systems at ambient temperatures. Some calibration and test processes useful in isothermal microcalorimetry conducted at ambient conditions are listed below. Notably, no dependable test reactions seem to be available in the important areas of sorption processes (adsorption and absorption of gases (vapours) and solutes on solid materials).

3.1. Neutralization processes

Neutralization processes have often been used in tests and calibration of calorimeters. The neutralization of strong aqueous sulphuric acid with an excess of aqueous sodium hydroxide solution has been recommended as a reference reaction system in macro reaction and dissolution calorimetry [6,7]. In microcalorimetry normally very dilute reagents are used which in neutralisation experiments may lead to problems with contamination of CO₂. The two pK_a values for carbonic acid at 25°C are 6.37 and 10.25 and both dissociation steps have low enthalpy values, compared to the high ionization enthalpy for water and for many buffers used in such experiments. In order to minimize the extent of reaction between the acid and the carbonic acid ions, the solutions should be freshly prepared from CO₂-free water, and be protected from uptake of CO₂.

Another problem which can disqualify neutralization reactions as test and calibration processes, in particular when flow mixing vessels are used, should be pointed out. When one reaction component is mixed with a large excess of the other component, the neutralization reaction may go to completion even if the mixing process is poor. Neutralization test reactions, in the way they normally are conducted, may give correct results even if the calorimetric vessel due to inferior mixing properties may not be suitable in studies of other mixing processes.

With a microcalorimetric vessel made from glass or metal there can be significant heat effects from the adsorption of ions by the walls of the vessel [8,9]. This effect can be avoided by equilibrating the vessel with the appropriate final salt solution prior to the measurement. It should also be kept in mind that even high quality stainless steel is easily corroded by dilute hydrochloric acid. When steel vessels and steel injection tubes are used it is safer to use nitric acid. For the

reasons given above, it is felt that neutralization processes should be used with great care in test and calibration work on the microcalorimetric level. The use of the aqueous dissolution/dilution processes of propan-1-ol in water (below) is normally a better choice.

3.2. Aqueous dissolution/dilution of propan-1-ol

The aqueous dissolution and dilution of propan-1-ol serves as a convenient test and calibration process [2–4] and has been much used in our laboratory. For microcalorimetric applications at ambient temperatures heat quantities evolved in the dissolution process tend to be too large and the dilution of 10% (w/w) propan-1-ol is then more useful. Revised enthalpy values for the propan-1-ol-water system have recently been reported [10].

3.3. Dilution of aqueous solutions of sucrose

The dilution of aqueous sucrose solutions, over a wide concentration range, has been used extensively in tests of microcalorimeters where two liquids are mixed. Concentrated sugar solutions (15–25%) are quite viscous and, especially in flow calorimeters, are not easy to mix with water. This dilution process is, therefore, particularly useful as a test of the mixing efficiency. Dilution enthalpies can be predicted by equations valid for the molality ranges $m_{\text{initial}}=0.1\text{--}2.0$ and $m_{\text{final}}=0.01\text{--}0.2$ mol kg⁻¹, respectively, and the temperature range 20–40°C [2,4]. The accuracy is estimated to be about 1%. (In Eq. (5) of reference [2] m_{initial} and m_{final} were incorrectly given in reversed order).

3.4. Dissolution of slightly soluble liquids in water

We have extensively used benzene as a test and calibration substance for aqueous dissolution processes involving slightly soluble liquids (flow systems) [11]. Only a few µl of benzene are used per experiment but the health hazards with this substance makes it less attractive as a reference material. Other liquids with low solubilities in water such as octan-1-ol [12] and some esters [13] have well known values for $\Delta_{\text{sol}}H$ and $\Delta_{\text{sol}}C_p$ could be more attractive as test and calibration substances.

3.5. Determination of excess enthalpies of mixing of organic liquids

When enthalpies of mixing are determined for organic liquids where one or both have a significant vapor pressure, it is necessary to conduct the measurement without gasphase and flow mixing microcalorimeters have, therefore, been much used in this area. Two mixing processes have been recommended as reference systems in macrocalorimetry and should also serve on the micro level [6,7]: cyclohexane+hexane (particularly for endothermic processes) and 1,4-dioxane+tetrachloromethane (particularly for exothermic processes). Use of the latter system is discouraged for health and safety reasons. It can be substituted by the water+ethanol system for which excellent data are available [6,7].

3.6. Processes initiated by dissolution of a solid

Dissolution of *tris* [tris(hydroxymethylamino-methane)] in 0.1 mol l⁻¹ aqueous HCl has been used extensively in tests of macrocalorimetric reaction and dissolution calorimeters [14,15] and is useful also in microcalorimetry. (Note that the HCl solution will corrode stainless steel vessels and injection tubes common in microcalorimetry). Commercially available analytical quality of *tris* is normally adequate for use as a calorimetric standard material, but it can easily be purified by recrystallization from aqueous methanol followed by 'mild' crushing, screening and storage for some time under vacuum [14]. Extensive grinding might lead to significant mechanical strain in the crystalline material [16].

The dissolution of KCl has been much used in tests of reaction and dissolution calorimeters [6,7] but a rather large spread in the reported values is found. It has been concluded [15] that the most consistent results have been obtained from samples that had been dried at temperatures above 300°C.

Dissolution of adenine in water has been used as a test process for slightly soluble solids [17].

3.7. Dissolution of gases in water

The enthalpy of dissolution of oxygen in water has been accurately determined for the temperature range 15–35°C. The process has been extensively used in our

laboratory for the calibration of dissolution microcalorimeters for slightly soluble gases [18].

3.8. Ligand binding processes

Thermodynamic characterization of ligand binding reactions using micro-titration calorimetry is particularly important in coordination chemistry and in biochemistry. It is frequently possible to determine, simultaneously, both the enthalpy change and the equilibrium constant (and thus, the standard Gibbs energy and entropy changes for the binding process) [1]. In such work it is often important to have available a process suitable for the test of the overall experiment, i.e. the calorimetric measurements as well as the calculation procedure. For this purpose we have proposed the binding of Ba²⁺ to 18-crown-6 (1,4,7,10,13,16-hexaoxacyclo-octadecane) in aqueous solution [2]. The reagents are stable and for most purposes commercially available in sufficiently pure form and the binding process is a clean 1:1 reaction.

Ligand binding processes in organic solvents are important, but no test reaction for such systems has been proposed.

3.9. Photochemical processes in solution

The photoreduction of potassium ferrioxalate in aqueous sulphuric acid solution (0.05 mol l⁻¹), i.e. the Hatchard–Parker actinometer process has found use as a test reaction in solution photocalorimetry [19]. The reaction mixture is corrosive against stainless steel but corrosion is prohibitive as the amount of Fe³⁺ reduced to Fe²⁺ is determined by analysis of the reaction mixture. Microcalorimetric vessels made by steel can be protected by teflon coating [19].

3.10. Generation of predictable long term thermal powers

In microcalorimetric work on biological systems and in the characterisation of slow decomposition of technical products, it is often important to have available a chemical test process producing a predictable thermal power over an extended period of time. The reaction system triacetin-imidazol-acetic acid-water mixed in different proportions has proved to be useful in this respect. Experimental details and data for

processes in the range of 6–100 μW are given in [2–4]. In flow calorimetry and for solutions agitated by stirring or rocking, a certain thermal power will result from friction effects which may be significant, in particular for solution mixtures giving low thermal power values. As yet, no attempt has been made to quantify such contributions.

3.11. Radioactive probes

We have found radioactive probes (Am, Monsanto) inclosed in stainless steel ampoules to be very useful for tests and calibrations of some types of vessels used for measurements of thermal power. However, the use and transportation of such probes, in particular across national borders, is restricted and they cannot be expected to come into common use.

4. Final remarks

There has been extensive methodological progress in the field of isothermal microcalorimetry and the technique has penetrated into several new application areas, for example in the pharmaceutical industry and in control of the stability of explosive materials. It is felt, however, that chemical test processes should be used much more frequently than today, many users do not pay attention to the risk that systematic errors might significantly influence the results. For some microcalorimeters a chemical calibration will lead to more accurate results than an electrical calibration.

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