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SYSTEMATIC ERRORS IN ISOTHERMAL MICRO- AND NANOCALORIMETRY

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Results of calorimetric measurements are easily impaired by different kinds of systematic errors. Isothermal micro- and nanocalorimeters are particularly sensitive to such errors, as the measured thermal powers are very low. In this paper the occurrence and effects of such errors are discussed: calibration errors, baseline errors, problems with evaporation/condensation, sorption, *pV*-work, gaseous reactants and different kinds of mechanical effects. Several numerical examples are given.

Keywords: *isothermal microcalorimetry, isothermal nanocalorimetry, systematic errors*

Introduction

The term 'isothermal microcalorimeter' may be defined as 'a calorimeter designed for use in the microwatt (μW) range, under essentially isothermal conditions' [1]. The term 'isothermal nanocalorimeter' has recently come into use and may thus be defined as 'a calorimeter designed for use in the nanowatt (nW) range, under essentially isothermal conditions'. However, in the present paper no sharp distinction is made between the two groups and usually only the 'micro' prefix is used.

Heat effects accompany practically all kinds of processes – they may be chemical, biological or physical. Calorimetry is therefore a universal tool in thermodynamics and is also useful in kinetic measurements and as a general 'process monitor'. A consequence of the non-specific nature of calorimetric measurements is that they are easily impaired by systematic errors. Examples of non-intentional processes leading to such errors are: evaporation, condensation, sorption, corrosion, various spurious reactions, and mechanical effects. Calibration of the instrument is another important source of systematic errors.

It is important to realize that the contribution from systematic errors to a measured thermal power (sometimes called 'heat flow') or heat quantity is often independent of the amount of material involved in the investigated process. In general, one can therefore expect that systematic errors will affect the results more seriously the smaller the investigated thermal power or heat quantity is.

Technical developments have made isothermal microcalorimeters very convenient to use. The theoretical and practical training of experimentalists in this field is therefore now often reduced to a brief introduction to an almost automatic instrument [2]. We believe that these developments have also led to a state where many new users of the technique are not well prepared to handle problems concerning systematic errors in experiments where very small thermal quantities are measured. Frequently, uncertainty values that are reported for results of such measurements do not appear to include estimates of systematic errors. To what extent can we then have confidence in the results?

In the following paragraphs some sources of systematic errors in isothermal microcalorimetry will be discussed, partly using simple numerical examples. The treatment will focus on experiments conducted at atmospheric pressure and in the temperature range 0–100°C. It will not be possible to cover all situations where systematic errors may occur. The primary goal is rather to increase the awareness of the problem among users, referees, and readers of reports.

Calibration errors

Isothermal micro- and nanocalorimeters are usually calibrated electrically, i.e. the instruments are standardised by the release of a well-defined electrical power or energy, in an electrical heater positioned in the calorimetric vessel or in its close proximity. It is desirable that the heat flow from the heater to the calorimetric sensor (usually a thermopile or a resistance thermometer) closely mimics the heat flow in the investigated process. However, it is sometimes a difficult task to design and position a heater with such

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properties. In fact, we believe that electrical calibration errors in the order of 5% are not uncommon for isothermal microcalorimeters in current use. It is therefore often important to use a chemical calibration method instead [1, 3]. Regardless which calibration method is used, it is desirable to check the calibration value, and the overall performance of the instrument, by use of a suitable test reaction. In order to allow a close comparison between chemical test and calibration reactions and the investigated reactions, it is important to have many different test and calibration reactions to chose from. Further development in this area is needed.

Baseline errors

If a calorimeter is left to equilibrate in isothermal surroundings, the baseline signal will adopt a characteristic value. For a thermopile heat conduction calorimeter the baseline value in the ideal case is zero. However, in practise it is common that the baseline value deviates significantly from zero, in particular when the measurement temperature differs much from that of the surroundings. Small displacements from the ideal baseline value should normally not be of any concern, as long as the value stays constant.

Changes in the baseline value can be of different origin and show different characteristics. Random baseline fluctuations ('noise') can be caused by thermal disturbances from the surroundings or, for example, from the mechanical effect of a stirrer or it may originate from the electronic system. Baseline fluctuations may give rise to random errors in short-term measurements, but they will often not cause problems in measurements over long periods of time, where positive and negative contributions will cancel out.

A drift or a stepwise change of the baseline, can give rise to severe systematic errors. If a continuous drift is small and regular, it may be possible to apply accurate corrections. Significant and unpredictable baseline changes between and during experiments will usually limit the accuracy by which a thermal power can be determined, for example in cell biology and in investigations of material stability. Stepwise baseline changes can often be seen if the calorimeter is disturbed, mechanically or by other means. For example, a change in the mechanical pressure on the thermopiles (in a thermopile heat conduction calorimeter) may cause baseline shifts when an insertion vessel is taken out or is returned into the instrument, or if some other mechanical function in the calorimeter is used. Sometimes, such changes are enough reproducible to be corrected for. By use of suitable control experiments it may be possible to obtain useful statistical estimates of their magnitude.

Evaporation and condensation

The enthalpy of vaporization of water at 25°C is about 44 kJ mol^{-1} [4] , which means that 1 μJ is absorbed per 0.4 ng of water evaporated. The vaporization enthalpy for water is comparatively high and water has a low molar mass, but the example indicates that practically no uncontrolled evaporation or condensation of any liquid can be allowed in experiments conducted on the nW level.

Gas perfusion

In many important types of experiment a gas is allowed to perfuse through (or above) a liquid in the calorimetric vessel. The gas flow must then contain a vapour with the same activity as that of the liquid phase, otherwise evaporation or condensation will take place in the vessel. Even minute differences in activity values can then give rise to very large thermal powers. Below is given an example where water is the liquid. The thermal power P (W) that is generated when a gas stream with the flow rate F (m^3 s^{-1}) changes its activity by Δa (Pa/Pa) is:

$$P = \frac{\Delta_{vap} H}{M} F \nu_S \Delta a \qquad (1)$$

M (g mol^{-1}) is the molar mass of water and v_s is saturation vapour content, 23 g m^{-3} [4] at 25°C. Assume that air, water activity 0.50 (a relative humidity of 50%) is perfused through the vessel at a rate of 10 mL h^{-1}. The vessel is charged with a wet biological sample where the water activity is 1.00 (the gas leaving the system is thus assumed to be humidified to a water activity of 1.00). The resulting cooling power will then be about 80 mW, i.e. often many times larger than the heating power of an investigated biological sample.

Clearly, it is in practice necessary that the gas flow entering the calorimetric vessel has virtually the same activity as that of the liquid in the vessel. One may come very close to that condition by passing the gas through a humidifier (usually a thin tube with the inner wall wetted with a solution of the requested relative humidity) that is placed in the thermostated part of the calorimeter i.e. having the same temperature as that in the calorimetric vessel [5].

In some cases it can be possible to perfuse intermittently and conduct the measurement when there is no flow, i.e. when the system has reached vapour pressure equilibrium.

Diffusion

Evaporation or condensation can also take place by the diffusion of vapour from a calorimetric vessel to

the ambient air. For example, diffusion can be effected through a minute hole or a leaking seal in the vessel, or through a tube used for the purpose of keeping the vessel open to the atmosphere. Further, significant flows of vapour, from water and organic liquids, may pass through various polymer materials used in the design of calorimeters.

Equation (2) shows the expected thermal power caused by diffusion of water vapour through a narrow metal tube, which connects the gas phase in a calorimetric vessel to the atmosphere.

$$P = \frac{\Delta_{vap} H}{M} \frac{\pi R^2}{L} D \nu_S \Delta a \qquad (2)$$

The inner radius of the tube is R (m) and its length is L (m). D is the diffusivity of water vapour, $25 \cdot 10^{-6}$ m^2 s^{-1} in air at 25°C [6] and Δa (Pa/Pa) is the difference in water activity between the calorimetric vessel and the ambient air. As earlier, M (g mol^{-1}) is the molar mass of water and v_s is the saturation vapour content, 23 g m^{-3} at 25°C. Assume that R and L are 0.3 mm and 0.3 m, respectively. Further, assume that the relative humidity in the air surrounding the calorimeter is 50% and that a biological tissue, kept wet by an aqueous medium (water activity 1.00), is contained in the vessel. According to Eq. (2) we can expect a cooling power in the vessel of about 0.7 μW.

The thermal power P will rapidly increase with increasing inner diameter of the tube. Thus, if R would be 3 mm the expected value for P would be about 70 μW. Diffusion of this kind can often be prevented, or at least decreased, by wetting the inside of the tube or by placing a wet object inside the tube, see for example [7].

In titration calorimetry a significant loss of titrant may take place by diffusive mixing between titrant near the tip of the syringe and the solution in the vessel. The effect can be particularly large if the titrant solution has a very low viscosity, for example methanol. To avoid this effect it is common to initiate each run with a small 'throw away' injection, but these problems can often be minimized by use of very thin syringes and short equilibration times. Another problem with the first injection step is that with some titration techniques the volume of the first injection step is low due to backlash in the pump screw mechanism. Such errors, which can be much larger than those caused by diffusion [8], are avoided by making a pre-injection outside the calorimetric vessel.

Changes of activity

It is common that injection of a pure liquid or a solution into a calorimetric vessel charged with some liquid will lead to a change in the vapour pressure of the vessel content and thus to changes in composition of its gas phase. As an example, consider an experiment where 5 μL of a dilute aqueous sucrose solution, concentration 10 mmol L^{-1}, is injected into a vessel containing 3 mL of pure water. The gas phase is 1 mL and the experimental temperature is 25°C. The initial water vapour pressure is 3170 Pa [4]. Application of Raoult's law suggests a vapour pressure change of about 1 mPa or a condensation of 0.4 pmol of water, corresponding to an enthalpy change of about 20 nJ, which may be significant on the nW level.

Sorption and adhesion

Vapour sorption

Related to the previous section on evaporation and condensation is the sorption (adsorption, absorption) of vapours on surfaces and materials. In an illustrative example reported by Paulsson *et al*. [9] exothermic peaks from water vapour adsorption are seen when the relative humidity of a perfusion gas is step-wise increased in an experiment on metal corrosion. We have also seen thermal effects of the absorption of water vapour in O-rings and one may expect that such effects may be even larger for organic solvents.

Sorption in the liquid phase

In experiments where very small thermal quantities are measured it is important to be aware of unintended sorption on the walls of a calorimetric vessel or in the flow lines of a flow calorimeter. Metal and glass surfaces can adsorb significant amounts of material, e.g. ions and proteins. Such effects can give rise to very large systematic errors; both from unexpected sorption enthalpies and due to the loss of materials in the flow-lines of a flow vessel.

Benzinger and Kitzinger [10] reported an illustrative example of ion sorption in a glass vessel in their early microcalorimetric experiments involving neutralization of NaOH solution with HCl at concentrations in the micromolar range. They noted an excess heat evolution if a cleaned calorimetric vessel was used. The effect disappeared if a small amount of NaCl was added to the NaOH solution prior to the addition of HCl solution. It was concluded that the sorption sites for Cl^- on the glass wall became saturated by the addition of NaCl. Similar effects have been observed in our laboratory, in experiments with different potassium salts using an 18-carat gold vessel [5].

The wetting of a dry surface in a calorimetric vessel by water or other solvents can also give rise to significant effects. A general method to avoid such sorption effects is to wet the surface before the experiment with the same liquid as will be used in the measurement [5].

Cell adhesion

The adhesion of living cells to the walls and to the flow-lines of microcalorimetric vessels can lead to large systematic errors that normally are difficult to correct for. The tendency for cell adhesion differs between different kinds of cells and between different surfaces. Depending on the experimental technique different types of errors may arise.

In experiments where a cell sample is injected into the reaction vessel it is possible that, for some types of cells, a significant fraction will be lost by adhesion to the injection tube. With flow-through vessels cells may be trapped by adhesion to the walls in the vessel and the thermal power from an erroneously large number of cells will then be measured [11].

When a batch vessel is charged with a cell suspension it is possible that a certain fraction of the cells will adhere to the walls of the vessel. It seems likely that the thermal power (the metabolic rate, the growth rate) will be different for cells in suspension and for cells adhering to the walls of the vessel. Further investigations on these problems seem warranted.

Metal corrosion

Metal corrosion is accompanied by large molar enthalpy changes that can lead to large errors, in particular in work conducted on the nW level. Calorimetric vessels are usually made from 'inert' materials like glass, stainless steel, Hastalloy or 18-carat gold, but even such materials can corrode. Ampoules with freshly machined surfaces may show some initial corrosion producing oxides that protect from further reaction.

Corrosion can be a problem for stainless (acid-proof) steel when weekly acid solutions contain halide ions. In such cases it can be useful to protect the inner surface of the vessel by polymers like polyethylene or teflon.

Pressure-volume work

An isothermal change of the pressure in a gas is accompanied by evolution or absorption of heat, followed by a heat exchange with the surroundings. If the pressure changes by Δp (Pa) in a volume of V (m^3), the pressure-volume work ΔpV (J) will be produced.

In many applications it is common to use injection vessels with a gas phase, for example in titrations and in work with living cells. In such cases the vessel is usually equipped with a narrow outlet tube ending outside the calorimeter. A problem encountered when measurements are made in the microwatt range and lower, is that changes in the atmospheric pressure will give a pressure-volume work in the vessel. As an example, it is not uncommon for ventilation systems to keep the indoor pressure 50 Pa lower than the outdoor pressure. If the ventilation system then is turned off and the pressure will increase 50 Pa in a vessel with 1 mL gas phase, an exothermic heat pulse of 50 μJ is generated.

Because of changes in wind pressure, fluctuations in ventilation, and when doors are being opened and closed the pressure changes all the time in most laboratories. This can give severe baseline fluctuations in measurements conducted in the nW level. Although this will often have the character of a random error, it can limit the usefulness of such measurements. The pressure-volume work inside a calorimetric vessel can be avoided if the calorimeter is pressure-sealed or if the vessel is completely filled with liquid.

If a liquid is injected into a vessel with a gas phase open to the atmosphere (usually by a thin tube) corresponding volume of gas will leave the vessel. However, in a closed vessel, or if the outlet tube non-intentionally is blocked, injections can be accompanied by large compression enthalpies that may ruin most microcalorimetric experiments, if no correction is applied. As an example, when 5 μL of liquid is injected into a closed vessel where the gas phase is 1 mL, the compression enthalpy will be about 500 μJ (assuming that the vessel content as well as the injected liquid has zero vapour pressure; with real liquids the process will also include vapour condensation as a result of the compression).

Gaseous reactants (O_2, CO_2)

If a gaseous compound reacts in a calorimetric vessel with a solute or a suspension it is important to identify the overall process that is measured. Will the reacting gas quantitatively be transferred from the gas phase or will the amount of dissolved gas change significantly during the course of the experiment?

Consider an aerobic experiment with living cells, which is initiated by injection of a cell sample into the vessel, which is partially filled with an aqueous medium. The gas phase, open to the atmosphere, contains air. At the start of the experiment it can be assumed that the medium is saturated with air. As the cells start to consume the dissolved oxygen, its concentration will decrease unless it is replaced by dissolution from the gas phase. We may then have two extreme cases: oxygen from the gas phase may dissolve as fast as it is consumed in the liquid phase or no significant amount of oxygen is dissolved during the experiment. Assuming that the nature of the biological process will be the same for the two cases, the difference between their enthalpy changes will be equal to

the enthalpy of dissolution of oxygen in the medium. This value is not known, but the value for pure water may be used as an approximation, 12.1 kJ mol^{-1} [12]. That value should be compared with 470 kJ mol^{-1}[12], a representative value for an aerobic biological process, per mol of oxygen. The enthalpy difference between the two cases would thus be less than 3%.

The formation of carbon dioxide in aqueous solution or in biological oxidation processes can be difficult to handle as aqueous carbon dioxide solutions easily become over-saturated. However, the amount of gas involved in experiments conducted on the nanocalorimetric level is very low. It will therefore often be possible to work entirely with dissolved gases, thereby avoiding the problems just discussed.

Bubble formation

Perfusion vessels are often completely filled with liquid, and the same is true for some titration vessels. When liquid is injected into such vessels a corresponding volume of the vessel content will leave through an outlet. Obviously, it is then important that inlet and outlet tubes are well separated in order to avoid that part of the injected aliquot will leave the vessel before it has become mixed with the vessel content. It is also important that gas bubbles are not formed in the vessel during an experiment, as they will become saturated with vapour, thus causing an evaporation effect, which must be accounted for when the result is reported.

Air bubbles can be formed in a vessel if it is charged with a liquid in equilibrium with air at a lower temperature than that of the vessel or if the liquid in the vessel is oversaturated with the gaseous compound. Gas bubbles can also be formed in the vessel as a result of a reaction. For example, when biological systems are studied it is common that carbon dioxide, or oxygen in case of plant tissue, is released.

As an example, assume that a small air bubble, volume 1 μL, is formed in an aqueous solution at 25°C and 1 atm. The evaporation will cause an endothermic enthalpy change of 56 μJ, i.e. much more than can be accepted in experiments on the nW level.

Gas bubbles, formed in a vessel or supplied to the vessel, may also cause other problems. Part of the reaction solution will be replaced by gas and thus less material than anticipated will be available, causing a systematic error. Further, gas bubbles in a flow line or in a vessel (flow vessel or stirred batch vessel) will often cause disturbances on the level of several μW. In some cases reaction vessels are put under pressure in order to suppress the formation of gas bubbles.

Mechanical effects

Experiments with test reactions are often useful in order to establish if a measurement problem relates to the investigated reaction or if it can be traced to, for example, some malfunctioning mechanical part of the instrument. However, some mechanical properties of an instrument may lead to errors in a measurement even if the instrument functions as intended. A few such problems are discussed below.

Breaking of glass ampoules and opening of valves

In 'macro calorimetry' it is common to initiate a reaction by breaking a glass ampoule or by opening a valve. The accompanying heat effects are normally by far too large (typically in the order of 50–100 mJ), and too irregular to be acceptable in microcalorimetry.

Stirring

When a liquid in a calorimetric vessel is stirred a thermal power due to friction in the liquid will be released. The effect will rapidly increase with increasing stirring rate and increasing viscosity of the medium. It will also depend on the design of the stirrer. Correspondingly, in a flow vessel the thermal power due to the liquid friction will increase with increasing flow rate and increasing viscosity of the medium. It will also depend on the design of the flow vessel; narrow channels and constrictions in flow mixing vessels (useful for the mixing efficiency) will increase the friction effect.

Such friction effects are typically significant on the μW level, but can often be considered as constant and be treated as a constant baseline displacement. However, in some cases the thermal power due to friction effects will change and may cause large systematic errors. For example, when polymerization processes are studied, and in microbial growth experiments, it is common that the viscosity of the reaction media will increase significantly.

In experiments with suspensions it is sometimes not possible to arrange for an efficient stirring (or agitation by other means). In case of metallic or other heavy inorganic particles unrealistically high stirring rates are required to keep the particles in suspension. In such cases the use of rocking or rotating vessels can be more efficient. However, such agitation techniques will often give rise to large and irregular heat effects, which might exclude their use on a sub-microwatt level.

If living cells are allowed to sediment one must count on a difference in their metabolism (growth) compared to that for cells in suspension. The diffusion rates

for nutrients and for compounds that are excreted from the cells are lower in the sediment than in solution, creating unfavourable conditions. For example, the metabolic rate usually depends much on the pH of the medium. In particular for cells that release lactic acid, the pH will be lower in the sediment than in solution, creating unfavorable conditions.

'Bench experiments'

For instruments where insertion vessels are used, it is often useful to conduct experiments outside the calorimeter, using dummy vessels made from transparent material (glass, plastic). It is then possible to visually inspect the performance of a stirrer or an injection device etc., and thereby avoid systematic errors. The flow pattern following an injection can be made visible by use of a coloured solution. Likewise, movements in a liquid can often be studied during continuous perfusion of liquid or gas, if some material with a suitable density, e.g. cellulose fibres, are added to the vessel. The tendency for sedimentation of a suspension can be studied for different stirrers using different stirring rates.

Relaxation effects

Most calorimetric vessels are sealed with polymer seals like o-rings, rubber septa or Teflon seals. Polymeric materials emit heat when they are compressed or stretched, which may cause slowly declining thermal power signals due to 'relaxation effects' in the material. Similar effects can be noted in many metals, e.g. in steel. Sometimes relaxation effects decline very slowly and may then be treated as constant baseline displacements. In other cases relaxation effects may cause an unpredictable decline of the baseline that is difficult to correct for.

Conclusions

Microcalorimeters are often used to monitor complex or poorly known processes, for example biological and technical processes. As discussed above, there are many possible sources of errors in such measurements. To reduce the risk of generating erroneous results, we propose that users of micro- and nanocalorimeters:

- Evaluate possible sources of errors before a measurements is performed. Make estimates of possible disturbances.
- Run standard reactions with well known heats and thermal powers. For chemical systems there are a large number of recommended reactions available [1].
- In order to avoid erroneous conclusions from the calorimetric results, support them by specific analytical measurements. These may sometimes be conducted in parallel with the calorimetric experiment, or on-line when a flow calorimeter is used. It has recently been shown that electrodes (pH, O_2) and a spectrophotometric cell, can be positioned in a microcalorimetric vessel and be used without interfering with the measurement of a microbial system [13].

References

1 I. Wadsö and R. N. Goldberg, Pure Appl. Chem., 73 (2001) 1625.
2 I. Wadsö, Thermochim. Acta, 300 (1997) 1.
3 L.-E. Briggner and I. Wadsö, J. Biochem. Biophys. Methods, 22 (1991) 101.
4 D. R. Lide, Handbook of chemistry and physics. 1993, Boca Raton FL, USA: CRC Press.
5 C. Spink and I. Wadsö, Methods Biochem. Anal., 23 (1976) 43.
6 T. R. Marrero and E. A. Mason, J. Phys. Chem. Ref. Data, 1 (1972) 3.
7 J. Suurkuusk and I. Wadsö, Chemica Scripta, 20 (1982) 155.
8 L. S. Mizoue and J. Tellinghuisen, Anal. Biochem., 326 (2004) 125.
9 L. E. Paulsson, L. G. Svensson and C. K. Forsgren, Microcalorimetric methods for corrosion rate measurements, Application Note TM001, Thermometric AB, Järfälla, Sweden.
10 T. H. Benzinger and C. Kitzinger, in Temperature - Its measurement and control in science and industry, Vol. 3, Part 3, Ed. J. D. Hardy (1963), Reinhold, New York.
11 K. Levin, Clin. Chim. Acta, 32 (1971) 87.
12 E. Gnaiger and R. B. Kemp, Biochem. Biophys. Acta, 1016 (1990) 328.
13 P. Johansson and I. Wadsö, Thermochim. Acta, 342 (1999) 19.

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