Journal of Thermal Analysis and Calorimetry, Vol. 57 (1999) 275-281

TOWARDS MORE SPECIFIC INFORMATION FROM ISOTHERMAL MICROCALORIMETRIC MEASUREMENTS ON LIVING SYSTEMS

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Abstract

Calorimetry is an important thermodynamic and analytical method for investigations of living systems. The non-specific nature of calorimetry can be of advantage in such work, but will also make it difficult to interprete the results in sufficient detail. It is therefore of interest to combine calorimetry with specific analytical techniques. Work in this area is discussed with reference to the use of isothermal microcalorimetry for the characterization of living cellular systems in the pharmaceutical industry.

Keywords: isothermal microcalorimetry, living systems

Introduction

For more than 200 years calorimetry has been an important method for the characterisation of living systems [1]. Until a few decades ago 'whole body calorimetry' and combustion calorimetry were the most important experimental techniques in this area. In whole-body calorimetry the thermal power (the heat production rate) is measured continuously for rather small animals like guinea-pigs or larger, like humans [1, 2]. Normally, such measurements are accompanied by determination of the rate of release/uptake of CO_2, O_2 water, urine etc. Combination of the calorimetric results with such analytical data, together with known enthalpy of formation values for biomass, energy sources and metabolites (determined by combustion calorimetry), are used in discussion of energetics of the metabolic processes. Presently, there are only few groups active in this area. Today's calorimetric work on living systems is mainly conducted by use of isothermal microcalorimeters and, in the field of biotechnology, by bench scale instruments.

The term 'isothermal microcalorimeter' is commonly used for calorimeters designed for work in the microwatt range under essentially isothermal conditions (in contrast to differential termperature scanning calorimeters, DSC). Isothermal microcalorimeters are employed in thermodynamic investigations but are also much used as general analytical instruments, for example in the characterization of stability and incompatibility of substances and materials of pharmaceutical and of technical importance.

The design of isothermal microcalorimeters and of experimental procedures have been much improved during the past decades, resulting in several new application

1418–2874/99/ $ 5.00
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areas. Much of that development work has been conducted with reference to applications in the field of living systems: human and animal cells and tissues, small animals (in particular small aquatic animals), plant tissues and microorganisms. A large part of the method work has been directed towards practical goals in areas like clinical and pharmacological analysis, ecology, agriculture, forestry and biotechnology. As an example, the heat effects accompanying cell-drug interactions (cells in suspension, cells adhering to different surfaces, tissue pieces) can now be conveniently measured using isothermal microcalorimetry, with a high accuracy and under adequate physiological conditions [3].

Isothermal microcalorimeters used for living systems

Different calorimetric principles are employed in isothermal microcalorimetry, but for instruments used in work on living systems thermopile heat conduction calorimeters are the most common type, for reviews see [4, 5]. In such instruments heat released (or absorbed) in the reaction vessel is allowed to flow to (or from) a surrounding heat sink, usually consisting of an aluminum block. A thermopile positioned between the reaction vessel and the heat sink serves as a sensor for the temperature difference causing the heat flow. For slow process the thermal power, P, produced in the reaction vessel is proportional to the temperature difference between the vessel and the heat sink and thus to the thermopile potential, U. This is the property measured by the calorimeter.

$$P = \varepsilon U \tag{1}$$

The proportionality factor, ε , is the calibration constant for the instrument. For processes where the heat flow will change fast on a time scale indicated by the time constant for the calorimeter, τ, the thermal inertia of the calorimeter will lead to a distortion of the calorimetric signal and a more complex relation should be used. The Tian equation

$$P = \varepsilon(U + \tau \mathrm{d}U / \mathrm{d}t) \tag{2}$$

derived under the assumption that temperature gradients in the calorimetric vessel are small, is often adequate.

Microcalorimeters are usually designed as twin instruments. Modern thermopile heat conduction calorimeters are normally equipped with semi-conducting thermopiles (‘Peltier effect plates’) which have a relatively large thermal conductance. The temperature difference between reaction vessel and the heat sink will therefore normally be small. In work with living cellular systems temperature differences are typically less than a few mK and measured processes can be considered as isothermal.

A non-specific technique

Calorimetry is a non-specific method and as an important benefit it has a very broad application range. It is sometimes useful to employ completely unspecific methods when complex or poorly known processes are monitored. It is then more likely that unknown or unexpected reactions will be recorded compared to cases

where highly specific methods are used. It is also important that calorimeters often can be used as continuous process monitors without interfering with the measured process. Further, it is rare that a process or a part-process has zero enthalpy change. A calorimeter with a high sensitivity and a low value for the time constant will thus have a very high resolution of consecutive events of complex processes, such as those in living cells and tissues. However, a calorimetric signal will not identify a measured process, or its different phases. For example, it is not possible to interpret in any detail the complex pattern typically obtained for microorganisms growing in a rich medium. As a typical example Fig. 1 shows a calorimetric record from growth of lactic acid bacteria in a defined medium [6]. It is seen that the growth curve is highly profiled reflecting a series of consecutive metabolic phases (due to changes of medium composition caused by the metabolic processes). The phases are recorded but are not identified.

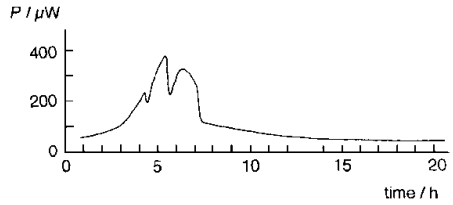

Fig. 1 Thermal power – time curve for a strain of lactic acid bacteria (*Str. diacetylactis*) grown under static and anaerobic conditions in a defined medium. Adapted from [6]

Many isothermal microcalorimeters are currently employed in the pharmaceutical industry, mainly for the characterisation of material properties and in investigations of drug-biopolymer complexes. However, the technique is hardly used at all in work with living cells or tissues. We find this surprising, but some problems connected with the technique should be recognised. Many scientists in the biological field lack the training to analyse results in terms of quantitative relationship between metabolic processes and heat. Further, the instruments are expensive, at least for users which do not plan to use them very frequently. However, the main problem is most likely that results of calorimetric measurements of complex reaction systems are difficult to interpret, on a molecular level. It is certainly possible to use the technique as an analytical tool solely on the empirical level. Most reports published in this field are of that kind and many of them clearly demonstrate interesting potential practical applications. Nevertheless, in the characterisation of properties of living systems much more information, analytically and thermodynamically, could be deduced from results of the experiments if they would be more specific, on the molecular level. The only way to achieve that is to combine the microcalorimetric experiment with specific analytical measurements.

Increasing the specificity

Several techniques have been explored where isothermal microcalorimetry and specific analytical methods have been combined. In such experiments the calorime-

ter serves as the 'master instrument' giving an overall account of the process, including events not recorded by the specific analytical measurements. If results of the analytical measurements can be expressed at the molecular level it is likely that the thermodynamic information from the calorimetric measurements can be fully utilised, see for example [7].

It is difficult to closely reproduce experiments on living reaction systems. Where possible, the calorimetric and the specific analytical measurements should therefore be conducted simultaneously and on the same reaction system – *cf.* the normal procedure in whole-body calorimetry. That experimental approach is not very common in isothermal microcalorimetric work on living systems. The analytical problems are usually more difficult in microcalorimetry than in whole-body calorimetry. In microcalorimetry very small amounts of substance are usually analysed and microcalorimetric experiments can easily disturbed by the analytical procedure.

Different types of analytical sensors can be positioned in a microcalorimetric reaction vessel and in flow or perfusion calorimetry it can be convenient to analyse gaseous or liquid fluids before or after they pass the calorimetric vessel. By use of light guides it is possible to employ a microcalorimetric vessel simultaneously for heat measurements and for light absorption measurements. With some precautions samples may be extracted from a batch vessel without seriously disturbing the calorimetric measurement. Below some examples of such techniques applied to living cellular systems will be reviewed.

Analytical sensors placed in the reaction vessel

It has been shown that electrodes can be positioned in microcalorimetric reaction vessels (volume 3–20 ml) and be used without causing noteable disturbances of

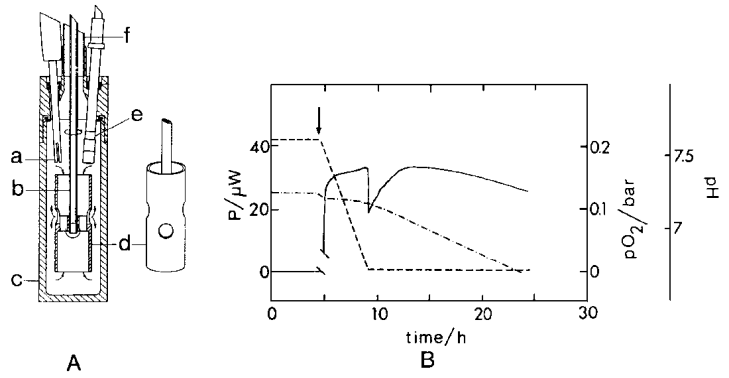

Fig. 2 Simultaneous determination of thermal power, *P*, oxygen pressure, pO_2 and pH
(A) Sample compartment (c, volume 3 ml) of a microcalorimetric vessel equipped with turbine stirrer (d), pH electrode (a) and oxygen electrode (e). Medium can be perfused through the hollow shaft (b) and will leave the vessel through the tube (f)
(B) Measurement of growing T-lymphoma cells. The sample compartment was completely filled with medium. The growth experiment was started by injection of 100 μl of cell suspension at the time indicated by the arrow. Adapted from [7]. Thermal power, *P*, —; pO_2, - - - -; pH, – · –

measurements conducted at the microwatt level. Figure 2A shows the sample compartment of an insertion vessel which was primarily designed for use in titration and perfusion experiments in biochemistry and cell biology [7]. A miniaturized pH glass electrode and a Clark cell for measurements of dissolved oxygen extend from the lid of the vessel into the sample compartment. For the function of the electrodes it is important that the vessel is well stirred. The stirrer indicated in Fig. 2A is of the 'turbine' type , which is particularly useful for vessels with a large length to width ratio, *cf.* [4, 5]. Figure 2B shows the record from an experiment with lymphoma cells using this microcalorimetric vessel. The vessel was completely filled with growth medium and no perfusion of medium took place. After an equlibration period a cell sample was injected into the calorimetric vessel resulting in a fast increase of the thermal power value to about 30 μW. The calorimetric signal continued to increase whereas the oxygen concentration and the pH value decreased. When all oxygen was consumed the thermal power rapidly decreased but already at a level of 20 μW the metabolism changed to an anaerobic state and the calorimetric signal increased during about 5 h. The following continuous decrease of the signal is expected from the decrease in the pH value. It is clear that the calorimetric curve is better understood through results of the electrode measurements. Further, the enthalpy value obtained for the oxygen consumption is needed for a thermochemical analysis of the experiment [7]. There exists a large number of different electrodes or biosensors which ought to be tested in experiments with isothermal microcalorimeters.

Schaarschmidt and Lamprecht have reported the use of light absorption in calorimetric vessels, both for determination of cell concentration [8] and in order to monitor chemical reactions in solution [9]. Mcllvaine and Langerman [10] used light guides to follow both heat and light produced by luminescent bacteria.

Figure 3 shows schematically a section through the sample compartment of a more complex reaction vessel which recently was designed in our laboratory [11]. The volume of the sample compartment was 20 ml. A turbine stirrer with propellers positioned above and below the 'turbine cylinder' was used (symbolised by a propeller in Fig. 3). As in Fig. 2B pH and oxygen electrodes were inserted through the lid. Light was introduced by means of a quartz rod (diameter 0.6 mm). After a 10 mm gap the light beam was conducted to a diode array spectrometer (positioned outside the calorimeter) and used to monitor cell concentrations.

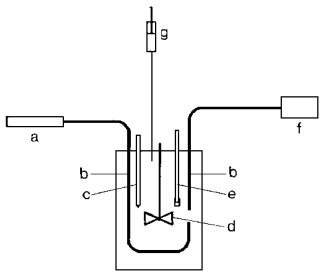

Fig. 3 Schematic picture of the sample compartment of a microcalorimetric vessel [11] for simultaneous determination of thermal power, pH, oxygen concentration and light absorption (light scattering), *cf.* the text. Light source (a); quartz rod (b); pH electrode (c); stirrer (d); oxygen electrode (e); diode array spectrometer (f); syringe (g)

Analysis conducted outside the calorimeter

There are several reports in the literature where flow microcalorimeters are connected on-line to specific analytical instruments. For example, in studies of small aquatic animals Gnaiger used oxygen electrodes positioned in the flow-line before and after a microcalorimetric perfusion vessel [12]. More recently Criddle *et al.* [13] used an assembly of two separate microcalorimeters and a pressure sensor in experiments with plant tissue (in the dark). One of the microcalorimeters was used for determination of heat produced by the tissue and the other was charged with a solution of sodium hydroxide. Carbon dioxide produced by the plant material diffused to the second calorimeter where it gave rise to a heat signal proportional to the rate of reaction with the hydroxide solution. The instrument assembly formed a closed system allowing oxygen consumption to be derived from results of the pressure measurements.

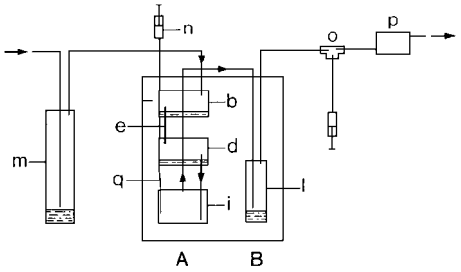

Fig. 4 Schematic diagram of the gas flow line of a double microcalorimetric system used for the simultaneous determination of thermal power and the release of carbon dioxide from a biological sample. (A) main calorimeter measuring thermal power of the biological object; (B) calorimeter for the determination of CO_2; (b) and (d) preequlibrium cups; (e) stainless steel tube; (i) sample compartment; (l) neutralisation chamber (charged with NaOH solution); CO_2 scrubber; (n) injection syringe; (o) gas sampling septum; peristaltic pump (p); injection needle. Reprinted from (q)[14]

An instrument from our laboratory, also used in measurements of plant tissue, employed a wet gas perfusion technique to force the carbon dioxide produced by the biological material in contact with a sodium hydroxide trap in a second calorimeter Fig. 4 [14].

There are several other techniques for extracting samples from microcalorimetric vessels which may be useful, for example microdialysis [15].

Conclusions

It has been shown that electrodes can be incorporated into microcalorimetric vessels designed for work with cellular material. Light can be introduced into calorimetric vessels for estimation of growth rates of cells in suspension. It is usually possible to extract gaseous or liquid samples for analysis from the flow line of a flow or perfusion vessel. Sometimes it is convienent to connect such vessels on-line to analyti-

cal instruments. We believe that such combined methods are needed in isothermal microcalorimetry in order to reach a wide use of the technique for the characterisation of living cells and tissues in the pharmaceutical industry.

References

1 J. A. McLean and G. Tobin, Animal and Human Calorimetry, Cambridge University Press, Cambridge 1987.
2 M. J. Dauncey, Thermochim. Acta, 193 (1991) 1.
3 I. Wadsö, Thermochim. Acta, 267 (1995) 45.
4 I. Wadsö, in Thermal and Energetic Studies of Cellular Biological Systems, ed. A. M. James, Wright, Bristol 1987, p. 34.
5 I. Wadsö, in Solution Calorimetry, ed. K. N. Marsh and P. A. G. O'Hare, Blackwell, Oxford 1994, p. 267.
6 T. Fujita, P. R. Monk and I. Wadsö, J. Dairy Res., 45 (1978) 457.
7 P. Bäckman and I. Wadsö, J. Biochem. Biophys. Meth., 23 (1991) 283.
8 B. Schaarschmidt and I. Lamprecht, Experienta, 29 (1973) 505.
9 B. Schaarschmidt, I. Lamprecht, T. Plessner and S. C. Müller, Thermochim. Acta, 105 (1986) 205.
10 P. Mcllvaine and N. Langerman, Biophys. J., 17 (1977) 17.
11 P. Johansson and I. Wadsö, to be published.
12 E. Gnaiger, J. Exp. Zool., 228 (1983) 471.
13 R. S. Criddle, A. J. Fontana, D. R. Rank, D. Peige, L. D. Hansen and R. W. Breidenbach, Anal. Biochem., 194 (1991) 413.
14 P. Bäckman, R. W. Breidenbach, P. Johansson and I. Wadsö, Thermochim. Acta, 251 (1995) 323.
15 R. G. Boutilier, Report at 10th ISBC Conference, Monte Verità, Ascona, Switzerland 1997.