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Isothermal microcalorimetry in applied biology

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Abstract

Techniques of isothermal microcalorimetry have been much improved during the past two decades. In addition to their use in fundamental research, applications of practical importance have been established in some areas. However, no significant use of isothermal microcalorimetry has yet been seen in practical applications of biology, despite many methodological studies reported from that area. The main problem appears to be that the sample throughput of isothermal microcalorimeters is low compared to other techniques used in that field. Further, the non-specificity of calorimetric signals is in some cases a serious limitation.

Significant progress has recently been made in the design of multi-channel isothermal microcalorimeters and in techniques where specific analytical methods have been combined with isothermal microcalorimeters. Some conclusions will be drawn with respect to the use of these techniques in applied work on living materials.

Keywords: Microcalorimetry; Multi-channel; Electrodes

1. Introduction

The term ‘isothermal microcalorimeter’ usually implies a calorimeter that is useful for measurements in the microwatt range, under essentially isothermal conditions. Such instruments are used in thermodynamic work, in kinetics and as analytical tools. Several important practical applications have been established, notably in the assessment of stability of drugs and explosives and in the characterisation of cement hydration [1].

For a long time it has been stated that microcalorimetric techniques are potentially useful in applications of practical importance in the field of living cellular systems. A few years ago, I examined the area with such statements in mind [2] and I found that a large number of methodological studies had been reported, in particular work related to medicine, biotechnology, agriculture, forestry and ecology. Important progress had been made in experimental techniques and essential problems had been identified. Many of the methodological studies were conducted by use of laboratory-made instruments, but commercial instruments useful for most of the experimental situations were available. However, no breakthrough could be seen in the practical use of isothermal microcalorimetry applied on living systems.

Instruments used in biological microcalorimetry were designed mainly for fundamental research and method developments, for which comparatively low sample throughputs can be accepted. However, applications of practical importance, often ‘process monitoring’, normally require a high sample throughput. Isothermal microcalorimetric measurements are bound to be slow in comparison with most other analytical techniques. An attractive approach to

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overcome, or at least to reduce this problem, is to use "multi-channel" instruments.

All living systems produce heat and, in principle, the heat evolution can always be measured calorimetrically, without any interference with the processes. The non-specific calorimetric signal from a complex reaction system is usually difficult to interpret on a molecular level, in the absence of more specific analytical information. However, the calorimetric signal does give an overall account of the complex process, which a specific analytical signal rarely will give. Further, it should be noted that calorimetric results, although non-specific, are expressed in terms of well-defined thermodynamic quantities, which significantly strengthen the obtained analytical information above that from most other non-specific measurements.

The non-specificity of calorimetric signals can be a limiting factor for the use of isothermal microcalorimetry in applied biology. A possible expedient to that problem is to equip the calorimetric vessel (or the flow line in the case of a flow microcalorimeter) with one or several specific analytical sensors, for example, electrodes. The non-specific calorimetric signal, in combination with specific information from the sensor(s), can form a very powerful analytical technique for the characterisation of complex systems [3].

Significant progress has recently been made in the design of multi-channel isothermal microcalorimeters and in techniques where specific analytical methods have been combined with isothermal microcalorimetry. Current multi-channel microcalorimeters and combinations between microcalorimeters and specific analytical instruments will be briefly reviewed. Some conclusions will be drawn with respect to their possible use in applied work on living materials.

2. Multi-channel isothermal microcalorimeters

Most isothermal microcalorimeters currently used in measurements of living systems are of the heat conduction type [1]. In such instruments the heat released in the reaction vessel is allowed to flow to a surrounding heat sink. A thermopile, positioned between the reaction vessel and the heat sink, is used as a sensor for the heat flow. Practically all isothermal microcalorimeters are designed as twin instruments and it is thus a differential potential that is measured. In a multi-channel heat conduction calorimeter several calorimeters normally share the same heat sink. One (or more) of the calorimeters is used as reference(s) for the other units, or the multi-channel instrument is made up by several twin calorimeters. Below a brief survey will be given of multi-channel instruments.

Already in the early 1970s a 50-channel microcalorimeter was developed at Instrumentation Laboratory Inc., (Lexington, MA, USA) [4,5]. The instrument, which was not described in any detail, was primarily intended for identification of microorganisms by use of their characteristic growth patterns. However, it was subsequently, shown that such growth patterns were not stable enough to be used for that purpose [6] and no further reports of that calorimeter seem to have appeared.

A few years ago Takahashi reported the design of a 25-channel instrument [7]. Glass vials (30 ml) used as reaction vessels are placed on thermocouple plates in contact with a large aluminium heat sink. One of the vessels is used as reference for the 24 'experimental' vessels. The instrument has been used in several studies of practical importance, e.g. antibacterial and antifungal actions of drugs [7], retardation of food spoilage [7,8], inhibitory effects of additives to cosmetics [9], decomposition of organic matters in soils [10] and investigations of seed germination [11]. A commercial version of this instrument is shown in Fig. 1 (19 + 1 channels, 50 ml vessels). The instrument is available from Laboratory of Biophysical Chemistry, Instrument Division, Keihanna Interaction Plaza, Seikacho, Kyoto, Japan.

An eight-channel twin instrument, based on a design by L. Wadsö [12], has recently become commercially available from Thermometric, Järfälla, Sweden, Fig. 2. Normally, 20 ml reaction vessels made from glass or stainless steel are used. The instrument was primarily designed for the characterisation of cement hydration and of other processes of technical importance that produce comparatively high thermal powers and for use in education. The instrument has also found use in studies of fermentation processes. The thermal power detection limit is stated to be $\pm 2\,\mu W$.

Thermometric has also started to market a 48-channel isothermal microcalorimeter that primarily was designed for the assessment of properties of technical products, for example, stability/compatibility of explosives and pharmaceutical compounds, Fig. 3.

Fig. 1. A 20-channel isothermal microcalorimeter of the heat conduction type. The instrument, based on a design by K. Takahashi, is marketed by Laboratory of Biophysical Chemistry, Kyoto. Glass vessels (50 ml) are placed in thermal contact with thermocouple plates, which are positioned in bores of the heat sink. One of the channels is used as reference.

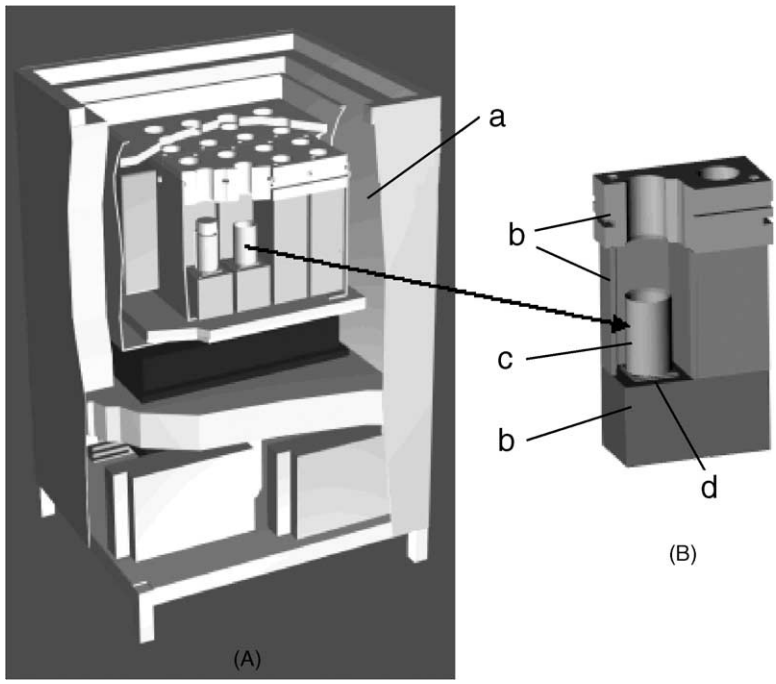

Fig. 2. An eight-channel isothermal microcalorimeter of the heat conduction type. The instrument, based on a design by L. Wadsö, is marketed by Thermometric. Each channel is a twin heat conduction calorimeter: (A) Cutaway view of the instrument; (a) air thermostat. (B) Detailed view of one channel; (b) aluminium heat sink; (c) vessel holder and (d) thermocouple plate.

Fig. 3. Thermometric's 48-channel isothermal microcalorimeter. Each channel is a twin heat conduction calorimeter. The two calorimetric units are positioned above one another, with the sample calorimeter at the top and thus accessible for the user. The tubular channels are submerged in a liquid thermostat.

In this instrument each channel is a twin calorimeter where the two units are positioned above each other. An experimental vessel made from glass or stainless steel, volume 4 ml, can be inserted into the top calorimetric unit. The 48 tube-shaped twin calorimeters are all inserted into a precise liquid thermostat (water or oil, 15–150 °C, short term variation $<\pm 50\,\mu$K, drift during 24 h within $\pm 100\,\mu$K). The two calorimetric units are separated by a small 'primary' heat sink that is in thermal contact with a surrounding steel tube. The liquid thermostat will thus serve as the main heat sink for all calorimeters. The thermal power detection limit for the twin calorimeters is stated to be 0.5 μW. A regulated change of the thermostat temperature allows the instrument to be used also in slow temperature-scanning experiments.

Hofelish et al. [13] recently reported from the ongoing work on a 100-channel twin microcalorimeter at The Dow Chemical Co. at Midland, MI, USA. Work is conducted jointly with Prof. Lee Hansen (Brigham Young University, Provo, UT), Calorimetry Sciences Corporation and Energetics Sciences

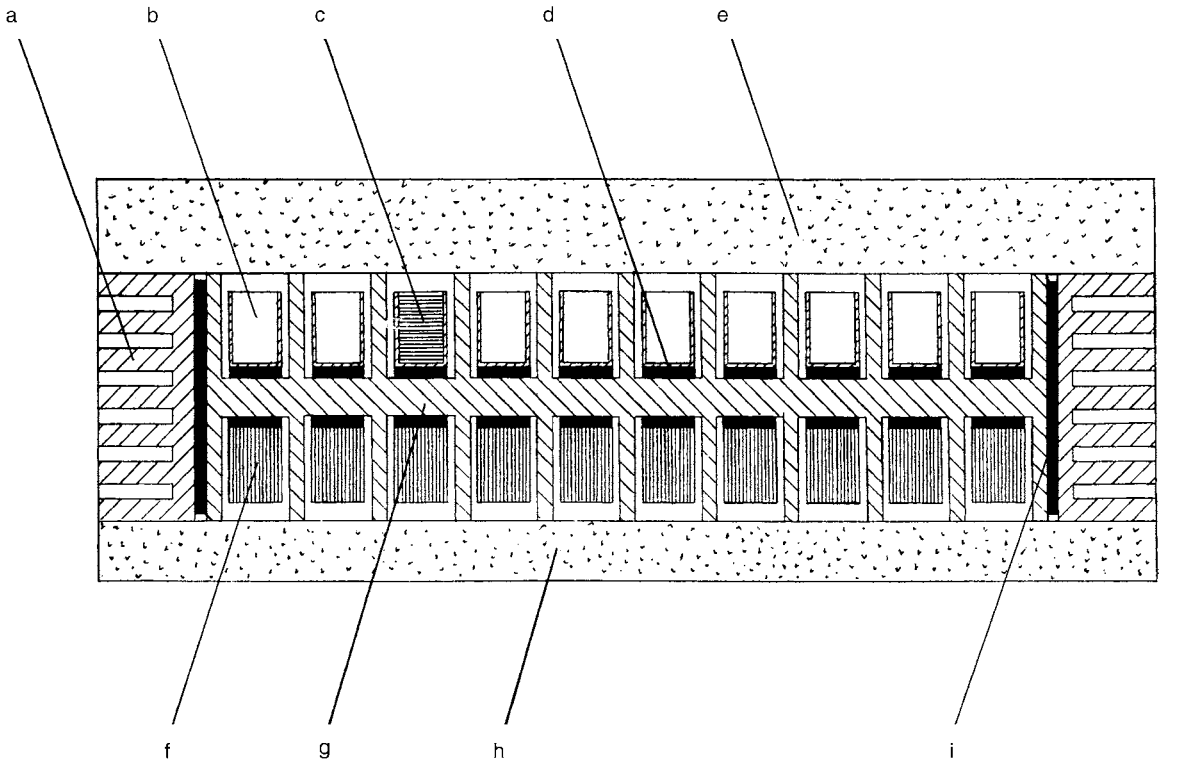

Fig. 4. A 100-channel microcalorimeter developed at Dow Chemical Co., Midland. The instrument can be used both in isothermal and scanning mode. Each channel is a twin heat conduction calorimeter. The two calorimetric units are positioned above one another, with the sample calorimeter at the top and thus accessible for the user. The figure shows schematically a section through the instrument: (a) sample well; (b) sample ampoule; (c) thermocouple plate; (d) reference well; (e) calorimeter block serving as heat sink; (f) thermocouple plates used for temperature control and (g) heat sink.

Corporation (Spanish Fork, UT). The instrument was based on a 16-channel prototype used for studies of metabolic rates in plant tissues [14]. The instrument at Dow is primarily intended to satisfy the needs for high throughput screening of samples in the chemical industry with respect to their stability/reactivity. Fig. 4 shows schematically a section through the instrument. It is seen that each sample calorimeter is individually connected with a reference unit positioned below the sample calorimeter. The instrument has a temperature range of 0–200 °C and can be used both in scanning and isothermal mode. Scan rates are up to 2 °C/min. The sample vessels are arranged in a 4×25 array operated by use of a robot. The screening capacity was stated to be 500 samples per day. Sample size is up to 0.7 ml and different types of vessel can be used.

A 48-channel calorimeter has been developed in the author's laboratory in co-operation with Dr. D. Hallén (Biovitrum, Stockholm) [15]. This multi-channel instrument is mainly designed for screening of samples of living cells and tissues. One of the 48 calorimetric units is used as reference. All vessels are introduced simultaneously into the measurement position. Vessel volume is about 1 ml. The thermal power detection limit is about 0.1 μW.

3. Towards more specific microcalorimetric measurements

It was pointed out in Section 1 that calorimetric records would be more informative if they were combined with results of specific analyses. It is often

not possible to reproduce the complex processes of living materials with a high accuracy. It is therefore, desirable that the calorimetric measurements and the specific analytical measurements are conducted in parallel on the same reaction system.

With a flow or perfusion calorimeter it is often possible to connect an analytical sensor on-line with the reaction vessel. Some types of analytical sensors, e.g. electrodes, can also be positioned in the calorimetric vessel and be used without significant interference with the calorimetric measurements. Normally, such vessels should be stirred or be agitated by some other means.

In many types of experiments with living materials oxygen concentration and pH are critical parameters. Oxygen starvation can take place, leading to a change of metabolic pattern or death of the living matter. A metabolic process will normally be accompanied by a change in the pH of the medium, which may lead to a significant change in the metabolic rate, even for a very small change in pH. For example, close to pH 7 the thermal power for red blood cells will decrease by 1.4% when pH of the medium is decreased by 0.01 pH unit [16].

In microcalorimetric experiments with cells or tissues suspended in liquid media it is normally quite easy to arrange for measurements of oxygen concentration (activity) and pH by use of electrodes [1,17]. For example, results from measurements of growing T-lymphoma cells in a perfusion vessel illustrate how the interpretation of the calorimetric curve is facilitated by the parallel measurements of pH and oxygen concentration [17]. If the experiments are conducted without perfusion it is possible to calculate the oxygen consumption.

In experiments with small aquatic animals Gnaiger [18] placed oxygen electrodes in the flow lines of a perfusion microcalorimeter. From electrodes positioned before and after the reaction vessel the oxygen consumption by the animals could be derived.

Criddle et al. [19] determined simultaneously carbon dioxide release, oxygen consumption and the rate of heat production from a plant tissue (in the dark) by use of two microcalorimeters. The assembly formed a closed system where the two calorimetric reaction vessels were connected by a tube. Carbon dioxide released from the tissue in one of the vessels could diffuse to the other vessel where it was absorbed by NaOH solution and measured calorimetrically. The change in pressure in the closed system was also measured, from which the oxygen consumption could be calculated.

Bäckman et al. [20] reported another type of microcalorimetric assembly where heat and carbon dioxide produced by plant tissue were measured simultaneously and continuously. Two (twin) gas perfusion microcalorimeters connected in series were placed in the same thermostatic bath. Humidified air was pumped through the reaction vessel containing the living tissue. The gas flow was then passed over NaOH solution in the reaction vessel of the second calorimeter where carbon dioxide released from the tissue was absorbed. By empirical calibration the carbon dioxide concentration could be calculated and be correlated with the measured heat production from the plant tissue. By use of a gas sampling septum in the flow line after the calorimeters discrete samples were extracted for external analysis.

Light can be introduced into microcalorimetric vessels under controlled conditions, e.g. by use of optical fibres or quartz rods. By such techniques the vessels can be used for the simultaneous determination of thermal power and the optical density, leading to values for changes in concentration of solutes or of suspended cells. The design and properties of a titration/perfusion microcalorimeter equipped with two electrodes (pH, O_2) and a spectrophotometer was recently reported [21]. The instrument was used in bacterial growth experiments [21].

4. Conclusions

It was pointed out in Section 1 that microcalorimetry has for a long time been thought of as potentially useful in the broad field of applied biology. Many “demonstration experiments” as well as a significant amount of systematic methodological work have been reported, but no application of practical importance has as yet been established. The low sample throughput and, for some applications, the non-specific nature of the calorimetric signal are believed to be the main causes for this lack of success. In this report several new multi-channel microcalorimeters suitable for work on living cellular systems have been described. Their sample throughput is one or more

orders of magnitude higher than one-channel (twin) microcalorimeters are capable of.

Techniques that will increase the specificity of microcalorimetric measurements were also discussed. It is felt that a combination of microcalorimetry with different specific analytical techniques can be very useful in many research applications. However, at this stage no attempts have to be made to incorporate specific analytical sensors into multi-channel microcalorimeters. Designs where batch or perfusion vessels that require stirring would be quite complex and probably less dependable than the multi-channel instruments discussed in this paper. Possibly, one attractive option would be to design multi-channel flow calorimeters with analytical sensors positioned in the flow lines.

To sum up, in several areas of applied biology methodological studies have been conducted by use of single channel isothermal microcalorimeters [22–24]. Examples, include work on microorganisms (e.g. effect of antibiotics, characterisation of soil and water systems), human or animal cells and tissues (e.g. interaction with drugs and drug candidates, compatibility with artificial materials, clinical analyses) and materials from plants (e.g. rate of biomass production, effect of stress factors, seed germination). An increased specificity of instruments used in applied work would be valuable. However, it is expected that already the present generation of multi-channel instruments will be important in these areas for quantitative 'process monitoring' of metabolic activities.

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