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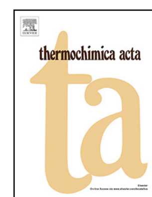

A well-plate format isothermal multi-channel microcalorimeter for monitoring the activity of living cells and tissues

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ABSTRACT

Design and properties are reported for a novel type of multi-channel isothermal microcalorimeter. It is equipped with 48 calorimetric units (channels) and is primarily intended for use as a monitor of the activity of living cells, tissues and small animals. Calorimetric vessels are positioned in a holder with the format of a 48-well microtiter plate. At most, 47 samples can be measured simultaneously; one vessel is then used as reference. The standard configuration is 32 sample positions using 16 channels as references in a twin calorimeter setup. The detection limit is then 0.1 μW. Sample volumes are usually 100 μl–300 μl. The 24 h baseline stability is typically 0.2 μW (room temperature variation ≤1 °C). The instrument was designed considering feasible uses in applied biology, especially in pharmaceutical and clinical laboratories and in environmental work. However, it can be employed as a general monitor of slow processes in different fields of biology and for non-biological systems, including accurate determination of their thermal powers (heat production rates). In the present report, properties of the instrument are characterized by chemical calibration experiments and in measurements of growth of bacteria and mammalian cells.

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1. Introduction

1.1. Isothermal microcalorimetry

Isothermal microcalorimeters are useful for measurements of thermal power (heat flow rate, heat production rate) in the μW range, or lower, under essentially isothermal conditions [1]. Currently, most such calorimeters are of the heat conduction type using semiconductor thermopiles (thermoelectric plates) as sensors for the heat flow between the calorimetric vessel and a surrounding heat sink. Normally, for such calorimeters a certain fraction of that heat flows to the surroundings by paths around the sensor and will thus not be recorded. However, by use of an adequate calibration technique, that partial loss of the heat released in the vessel will not cause any systematic error in the results.

Measurements of thermal power (heat production rate) of living organisms can be made without any labeling and under oxic or anoxic conditions. The technique can be applied to cells (in suspension, in sediments or attached to solid surfaces) tissues, small animals and plants [2–4]. The thermal power of living organisms can be monitored continuously, without any interference with the samples and over long periods of time (days, on the μW level) [2,5]. Furthermore, isothermal microcalorimetric techniques can have higher reproducibility and detectability than many other analytical methods used in measurements of a functional property of living organisms. Thus, isothermal microcalorimetry is an ideal technique in monitoring the activity of living organisms. However, the low rate of sample throughput for heat conduction calorimeters has limited their use in practical work. To some extent, this disadvantage can be balanced by the use multi-channel instruments by which several samples can be measured simultaneously. In such instruments the calorimetric units ("channels") are joined into one instrument where one (or several) of the channels is used as a ref-

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erence. The assembly is positioned in the same thermostated unit. Alternatively, multi-channel instruments are made up by a number of twin calorimeters that share one thermostated entity.

All metabolically active organisms produce heat, making isothermal calorimetry a general technique for monitoring their activities. In some cases it is desirable to supplement the calorimetric measurements with specific analytical methods. In a few cases, isothermal microcalorimetric vessels have been equipped with sensors for measurements of some specific properties. Johansson and Wadsö [6] reported a vessel fitted electrodes and a spectrophotometer allowing the simultaneous determination of thermal power, pH, oxygen concentration and cell density. Similarly the use of multi-channel DSC (used in isothermal mode and scanning mode) with modified vials accomodating optrodes was reported to allow oxygen measurments in combination with thermal power measurments [7,8]. However, no commercial multi-channel instrument fitted with specific analytical sensors has been reported yet.

In the early 1970s, a 50-channel microcalorimeter was developed by Boling et al. [9] that primarily was intended for use in the identification of microorganisms. Its design and use was reported in more detail by Russel et al. [10], but no further employment of their technique has been found; cf. the criticism of the method by Beezer et al. [11]. Graf et al. [12] reported the design and properties of a 10-channel instrument. It was used for studies of marine sediments using very large (70 ml) vessels. Takahashi's comparatively simple instruments, (called "multiplex calorimeters") [13], cf [5] have been used in several investigations in different areas of applied biology. Calorimetry Sciences Corporation (CSC), Lindon, UT, USA, marketed 4-channel microcalorimeters that could be operated both in isothermal mode and as temperature scanning instruments. These were used in many studies on living systems, especially on plant tissues, and insects [4,8,14].

A 4-channel twin microcalorimeter, based on an instrument designed by Suurkuusk and Wadsö [1,15], was developed by LKB Producter and by Thermometric (Stockholm, Sweden) to a series of commercial instruments called TAM (Thermal Activity Monitor). Different versions of the 4-channel microcalorimeter were followed by an instrument fitted with 48 twin calorimeters and a simpler instrument with 8 twin channels. Those instruments are currently much used as monitors of the activity of different types of living organisms and in studies of kinetic and thermodynamic properties of chemical and biological systems [16,17]. The TAM group of multichannel microcalorimeters are now produced and marketed by TA Instruments (New Castle, DE, USA).

1.2. Calibration experiments

In thermodynamic work on well-defined systems it is required that calorimeters are accurately calibrated. Although the obtained thermodynamic information is of importance [17–19] for biology, it is often disregarded by others to focus mostly on simpler descriptors of the heat production rate [20]. However, in order to allow a close comparison between results of different investigations it is important that the instruments have been appropriately calibrated and that calibration data are expressed by use of standard units and terminology. Calorimeters are usually calibrated by the release of electrical energy in a heater positioned in the reaction vessel or in its immediate surroundings. A certain fraction of the heat flow from the reaction vessels to the thermopile plates will flow to the surroundings by other paths and will thus not be recorded. In such calibration experiments it is important that the heat flow generated by the electrical heater closely mimics the heat flow caused by different reactions in the calorimetric vessel. However, for the isothermal microcalorimeter described in this report, and for several other designs, it is difficult to arrange for that condition. Preferably, a chemical calibration method should be used. A range of such calibration reactions have been developed to meet the needs for different types of reactions [1,21].

1.3. Monitoring the activity of living organisms

The thermal power, P *(also noted* Φ*)*, for a living system is related to its metabolic rate and may therefore be taken as a quantitative measure of its "activity". P is a strictly defined property, but for biological systems it is a complex property, that can be analyzed in detail if other parameters such as O_2 consumption or CO_2 production are measured as well thus allowing calculations of "heat per O_2" and "heat per CO_2". This can provide valuable insights on the metabolic pathways and their efficiency [17–19]. However, in many studies P is measured alone, and therefore it is often analyzed in less detail [20] and used only to indicate cellular growth. Single values, P_{exp}, should therefore be compared to the value of a reference (control) system, P_{ref}. The difference between P_{exp} and P_{ref} leads to the applicable relative activity value, P_{rel}. For example, the difference between P-values determined for a specific cell system with or without the presence of a drug (P_{exp} and $P_{ref,}$ respectively) can provide useful quantitative information about properties of the drug, without any further knowledge of the reaction system. One of the most promising uses of isothermal microcalorimetry in applied biology is in the field of drug discovery and development [22,23].

New compounds can be tested on the target cells such as bacteria, parasites, tumor cells as well as on a mammalian cell line (L6 or Vero cells are often used for cytotoxicity assessments) to compare drug activity and to determine the selectivity of the compound [24,25]. The specialty of isothermal calorimetry is that time of drug action and time to kill can be determined on a real-time basis in a simple and user friendly way.

P_{exp} for a certain type of cells or tissues from a patient with an active or untreated pathology, P_{pat}, is usually significantly different from the corresponding value for the same type of cells from healthy individuals, P_{health}, which then can serve as a reference value. However, the spread between values for P_{health} for different individuals is often too large to make the method useful for diagnostic applications [26]. Yet, if values for P_{pat} are determined for the same patient during a period of time, values for P_{rel} can be used as a prognostic value [27] and may then serve as a guide in drug treatments.

Investigations of the compatibility between organisms and different objects made from metal or organic polymers are important in tissue engineering and implant design as such materials are in contact with living tissue. Isothermal microcalorimetric techniques are judged to be useful in the characterization of such systems [28,29]. P_{exp} is then determined for the biological material in contact with the artificial material, in a suitable medium. In other experiments P_{exp} values of the tissue and of the artificial material (both in contact with the medium), are measured separately. The sum of these latter P_{exp} values will form the P_{ref} value.

2. Design of the 48-channel instrument

The instrument described below is a pre-production model of the commercial instrument CalScreener (SymCel Sverige AB, Spånga, Sweden), which was based on a prototype developed by Hallén and Wadsö at University of Lund, Sweden [30]. The SymCel instrument is shown schematically in Figs. 1 and 2.

The main parts of the calorimetric assembly consist of three individual aluminium blocks: the heat sink (k), with its 48 thermopile plates and two thermostated aluminium blocks (i, j). The heat sink is suspended in the cavity of the main block (j) by use of 4 plastic spacers. The upper part of the heat sink is divided into 48 pillars (l), in order to provide space for the electrical leads and to reduce

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