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Isothermal microcalorimetry near ambient temperature: An overview and discussion[1]

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Abstract

Isothermal microcalorimeters are employed both in thermodynamic work and as general analytical tools. Important application areas include ligand binding studies by use of titration techniques, sorption of solutes and vapours on solid materials, measurements of dissolution processes (particularly for slightly soluble compounds), assessment of physical and chemical stabilities and investigations of living systems: microorganisms, animal and human cells and tissues, small animals and plants tissues.

During the past 30 years much development work has been conducted in these areas in relation to instrument design and experimental procedures. At present, an important trend involves the combination of microcalorimetry with different specific analytical methods. © 1997 Elsevier Science B.V.

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1. Introduction

The term 'isothermal microcalorimeter' is often used for calorimeters which have a sensitivity in the range of one μW or better and which, in contrast to temperature scanning calorimeters, are operated under essentially isothermal conditions. Isothermal microcalorimeters are usually designed for work in a temperature range of approximately 10–80°C and are, in most cases, of the thermopile heat conduction type; nevertheless, the adiabatic and the power compensation principle are also employed. Like all calorimeters, they are used for measurements of thermodynamic properties, but for certain applications they are primarily employed in analytical work, often as 'process monitors'.

Non-specific methods, like calorimetry, are attractive from the point of view of their broad application range and they are particularly well suited for the discovery and quantitative assessment of unexpected or unknown phenomena. However, it is often felt that the inability of calorimetric techniques to identify the processes investigated is a serious limitation. It is therefore often important to combine isothermal microcalorimetry with specific analytical methods.

2. Some principles and design characteristics

2.1. Heat conduction calorimeters

The heat evolved (or absorbed) in the reaction vessel of a heat conduction calorimeter is conducted to (or from) a heat sink, normally a metal block surrounding the vessel. The heat flow is recorded by allowing a certain fraction of it to pass through a thermopile wall positioned between the vessel and the heat sink. At steady state, the heat production rate or the thermal power, P, is directly proportional to the

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thermopile potential, U,

$$P = \varepsilon U \quad (1)$$

The heat quantity, dq, evolved during the time dt is thus,

$$dq = \varepsilon U dt \quad (2)$$

where ε is the calibration constant.

In the ideal case, ε is equal to the quotient between the thermal conductance of the thermopile and the Seebeck coefficient for the thermopile material (see e.g. [1,2]). The fraction of the heat flow which does not pass through the thermopile is accounted for by the empirical determination of the calibration constant.

For an ideal heat conduction calorimeter the thermal power for a non-steady state process is expressed by the Tian equation,

$$P = \varepsilon(U + \tau dU/dt) \quad (3)$$

The time constant, τ is given by

$$\tau = C/G \quad (4)$$

where C is the heat capacity of the vessel and its content plus the heat capacity of half the thermopile, G the thermal conductance of the thermopile plus that of leads, mechanical supports and the surrounding air. The time constant for a heat conduction microcalorimeter fitted with a thermopile made from a semiconducting material is typically in the order of a few minutes. In practice, it is sometimes necessary to use an expression with more than one time constant [3,4]. Integration of Eq. (2) or Eq. (3) will give

$$q = \varepsilon \int U dt \quad (5)$$

provided that the initial and the final potentials are identical, usually close to zero. It may be noted that the calibration constant for a heat conduction calorimeter (the reverse of its sensitivity), is independent of the heat capacity of the vessel and its content. However, if the kinetics of a non-steady state process is derived (Eq. (3)), the heat capacity is taken into account by the value for the time constant. In the literature it is common that authors have used the simple Eq. (1) to express rates of processes, even in cases where dU/dt is significantly different from zero. This appears to be particularly common in reports on living cellular systems where, however, rate changes usually are small. With modern heat conduction microcalorimeters, output data can usually be obtained automatically in terms of thermal power values derived from Eq. (3) or by a more complex expression using two or more time constants [3,4].

A heat conduction calorimeter can often be used with several different vessels which are not permanently mounted in the calorimeter ('insertion vessels'), allowing the instrument to be used in widely different types of experiments.

Heat conduction microcalorimeters have rather large time constants, which can be a disadvantage. When a fast process takes place in the vessel of a heat conduction calorimeter, it will take about 7τ until 99.9% of the heat has leaked out to the heat sink. Together with base line observation periods, the measurement time may approach one hour and a multi-step titration experiment (cf. below) will thus require several hours. However, it has been shown that it is not necessary to wait until all heat from a previous reaction step has leaked out before the next addition of titrant is made (see e.g. [2]). By use of a 'dynamic correction' procedure based on Tian's Eq. (3), it is possible to shorten the time for a series of titration experiments by more than one order of magnitude, without any loss of accuracy (cf. Fig. 1).

Most isothermal microcalorimeters in current use are of the thermopile heat conduction type, e.g. the instruments produced commercially by CSC (formerly Hart Scientific), Setaram (Calvet type) and Thermometric (TAM microcalorimetric system).

2.2. *Adiabatic and power compensation calorimeters*

In an ideal adiabatic calorimeter there is no (net) heat exchange between the calorimetric vessel and its surroundings. Normally, adiabatic conditions are provided by an 'adiabatic shield' surrounding the calorimetric vessel. The heat quantity evolved during an experiment is equal to the product of the measured temperature change and the heat capacity of the vessel and its content. For semi-adiabatic instruments, often called 'isoperibolic calorimeters', a correction must be applied to account for heat exchange with the surroundings.

The enthalpy change for a process measured in an adiabatic calorimeter will refer to the isothermal

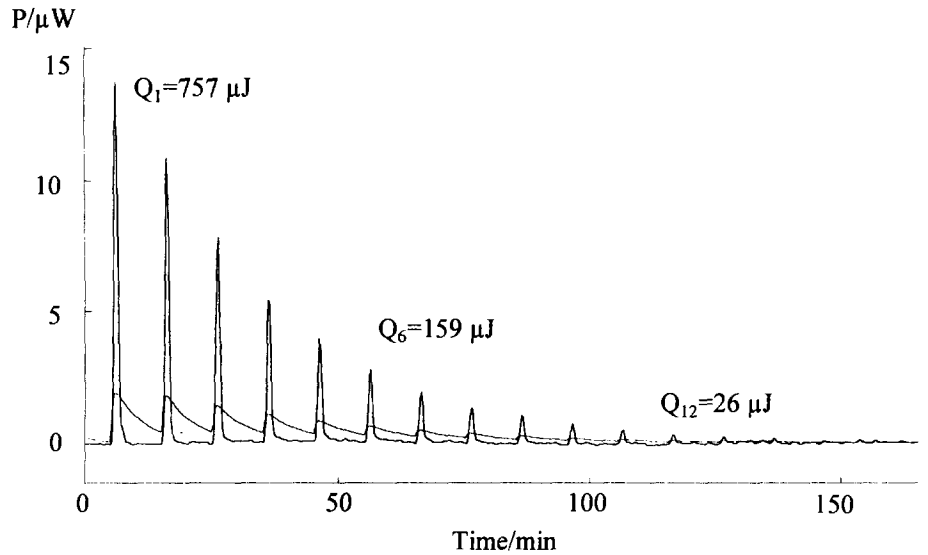

Fig. 1. Result from titration of ribonuclease by cytidine monophosphate using a commercial heat conduction microcalorimeter. (Thermometric, stainless steel vessel, volume 2 ml, $\tau = 200$ s). A 'dynamic correction' technique was employed to speed up the experimental curve, the curve with sharp peaks and clear baselines between the injections is the corrected curve. For some of the injections the corresponding heat quantities are indicated. The time between injections in a titration experiment involving fast reactions, can be reduced to ~5 min. (Courtesy Thermometric, Järfälla, Sweden).

process at the final temperature if the initial system is calibrated electrically before the process has taken place [5]. If the calibration is conducted on the reacted system the enthalpy value will refer to the isothermal process at the starting temperature. However, for an adiabatic microcalorimeter, not operating as a DSC, the temperature change during measurement of a single reaction step is often not significant when stating the absolute temperature at which the measurement was conducted.

When an endothermic process is measured by any type of calorimeter the accompanying temperature decreases, or the heat flow may be compensated for, by the release of corresponding electrical energy, or power, in the calorimetric vessel. For exothermic processes, the temperature can be kept constant and the heat measured by use of Peltier effect cooling: when an electrical current, I, is allowed to pass through a thermocouple plate, there will be a cooling effect on one side of the plate and a heating effect on the other side. With a thermopile positioned between the vessel and the surrounding thermostatic bath (or heat sink) and with the correct direction of the current, heat will be 'pumped' from the vessel to the surroundings. The cooling power, P_c, is

$$P_c = \pi I - rI^2 \tag{6}$$

where π is a constant and r the effective resistance value for the thermopile. The thermal power for the process is equal to P_c, provided that the temperature of the vessel is kept constant.

The titration microcalorimeter produced by Microcal (Omega), sometimes described as an adiabatic calorimeter, is equipped with an adiabatic shield but makes use also of a feedback system for the determination of reaction heats [6] – this instrument may be considered as a DSC running at a very low scan rate (≤ 0.1°C/h).

2.3. Design features

All types of microcalorimeters are normally designed as twin or differential instruments, where the 'reaction vessel' is used for the actual process and the 'reference vessel' is charged with an inert material. Regardless of the calorimetric principle employed, an optimal performance of the twin instrument requires that the heat capacity and the heat conductance of both

vessels and their contents be closely similar – a fact which, sometimes, is overlooked.

Isothermal microcalorimeters form a very heterogenous group of instruments designed to suit the experimental requirements at different types of processes. Measurements may be conducted as batch experiments, with or without agitation (stirring) of the content of the vessel, or as continuous flow experiments. In measurements of relatively fast kinetics, stop-flow techniques have been used [1]. Different experimental requirements call for very different reaction vessels, which sometimes are designed to form a modular system of 'insertion vessels', which can be easily exchanged [1,7].

In macro reaction/solution calorimetry, it is common to initiate a process by breaking a glass ampoule containing a reagent. In microcalorimetry, such techniques are usually not suitable as the heat of breaking is too unpredictable to allow an accurate correction. For fluid reactants, injection techniques comprise the most common method of initiating a process. They can be used with small heat effects and are suitable for automatic operation, e.g. in titration calorimetry. In several early microcalorimetric designs, bicompartment mixing vessels, operated by the rotation or rocking of the calorimetric assembly, were common. The addition of a solid substance in a microcalorimetric experiment is more difficult (cf. Section 3.2).

Several flow microcalorimeters have been reported where a process is initiated by bringing together two liquid reagents in a flow mixing vessel. Such designs have also been widely used in 'titration' experiments, but it is felt that batch injection techniques are generally superior. The mixing of a liquid reagent with a heterogenous system in a microcalorimetric experiment, e.g. a finely divided solid which tends to sediment, can be difficult. In such cases, 'old-fashioned' rotating or rocking assemblies can be the preferred instruments.

3. Application areas

Currently, the main application areas for isothermal microcalorimetry at ambient temperature and normal pressure are investigations of ligand binding processes using titration calorimetry, studies of sorption processes, measurements of physical and chemical processes in solids and in work on living cellular systems. Activities in these areas and a few other types of applications will be briefly discussed. Special attention will be given to developments which are believed to be of interest for the applied areas.

3.1. Stepwise titration calorimetry

The fraction of titrant reacting in a calorimetric titration step is determined by the (concentration) equilibrium constant for the reaction, K_c, and the heat quantity released is determined by the product between K_c and the molar reaction enthalpy (it is assumed that the experiment is conducted at constant pressure). From values for heat quantities evolved at the different titration steps, and assuming a certain stoichiometry for the binding reaction, both K_c and the molar enthalpy change may be derived by use of some minimization procedure. Commercial instruments are now usually supplied with computer programmes for the calculation of K_c and ΔH values. From Eq. (7) the corresponding entropy change can be calculated:

$$\ln K_c = -(\Delta H - T\Delta S)/RT \tag{7}$$

If the titration experiment is conducted at different temperatures, the change in heat capacity, ΔC_p, can be derived as follows:

$$\Delta C_p = \mathrm{d}(\Delta H)/\mathrm{d}T \tag{8}$$

For binding reactions in aqueous solution, ΔC_p values are of special interest in discussions on hydrophobic interactions [1].

When K_c is very high, the titrant will be almost completely consumed at all steps until the equivalence point has been reached. Such experiments will give information about the stoichiometry for the process and will directly lead to a value for the molar enthalpy change, but a value for K_c cannot be obtained. In some cases, it is possible to employ 'displacement titration technique' at which the process is divided into two energetically smaller steps and larger values for K_c may then be determined. For a recent example, see [8].

Titration microcalorimeters have been further developed during recent years [1]. To a large extent, applications are concerned with the binding of relatively small molecules to biopolymers, mainly the binding of inhibitors to enzymes. Such work has played an important role in biophysical chemistry

for more than 25 years and is presently gaining attention in the pharmaceutical industry as a significant tool in 'rotional drug design' [9]. Studies on binding of small molecules to macrocylic compounds is another active area for titration microcalorimetry [10]. To a certain extent, such techniques are used as model systems for processes involving biopolymers.

3.2. Dissolution and mixing microcalorimetry

The measurement of dissolution enthalpies, $\Delta_{sol}H$, for pure compounds into well-defined solvents are usually undertaken to obtain information about solute–solvent interactions. The difference between $\Delta_{sol}H$ values, determined for the same substance in different solvents, will give the transfer enthalpy for the compound between the solvents. Such values are of great interest in studies of, for example, biochemical model systems, where one solvent usually is water and different organic solvents serve as models for the interior of protein molecules, lipid environments, etc. Enthalpy and heat capacity changes for the transfer of molecules from the gas phase to infinite dilute solutions are of fundamental importance in solution chemistry. These quantities, expressing properties directly related to interactions between solute and solvent, are derived from differences between dissolution and vaporization data. Similarly, the partial molar heat capacity value for a compound, (A), at infinite dilute solution, $C^{\infty}_{p,A}$, is free from contributions from intermolecular forces between the solute molecules. $C^{\infty}_{p,A}$-values have proved to be of particular importance in studies of solute properties in aqueous solution, where 'excess' values often reflect hydrophobic hydration. C^{∞}_{p}-values are obtained from heat capacity measurements conducted on very dilute solutions or from the sum of $\Delta_{sol}C^{\infty}_{p,A}$ and the heat capacity for the pure solute.

In some cases dissolution calorimetry is used to obtain information about the state of solid materials. In the pharmaceutical industry, it is important to characterize the physical state with respect to polymorphic forms or the fraction of amorphous form present in a compound, or formulation of compounds [11] (cf. vapour sorption microcalorimetry, below).

In the late eighties, an intense development work took place in micro dissolution calorimetry [1,12], mainly to meet needs in work on biochemical model systems, in particular of simple, slightly soluble organic compounds in water and in other solvents. Instruments were developed for the determination of easily or slightly soluble liquids, slightly soluble gases as well as solids. Most of these instruments have the character of perfusion–titration vessels. Several of them are made to fit into Thermometric's microcalorimetric system (TAM), but they are as yet not available commercially.

Murphy and Gill have reported an interesting dissolution method for slightly soluble (wet) solids using a titration microcalorimeter [13]. Very recently a new microdissolution calorimeter suitable for measurements of easily or slightly soluble solid samples was developed [14].

There is still a need for fundamental dissolution studies which require microcalorimetric techniques not the least as a support for interpretation of results from titration calorimetric measurements of ligand binding processes. However, very few investigations in dissolution calorimetry are conducted at present, probably due to the lack of commercial instruments.

Ideally, the enthalpy of mixing of two liquids which do not react chemically is zero. Experimental values, which usually are significantly different from zero (excess enthalpies), are often determined by macro- or micro flow calorimetric techniques [15]. Results from a very large number of such experiments, which are of importance theoretically, and in chemical engineering, have been reported during recent years.

3.3. Sorption of vapours and solutes

Microcalorimetric measurements of (ad)sorption of vapours and solutes onto solids are of increasing importance, in particular, in applied areas. Frequently, the same calorimeter is used for both types of experiments, but the reaction vessels are different.

3.3.1. Vapour sorption

During recent years, microcalorimetric measurements of vapour sorption on solids has become a routine tool in many pharmaceutical laboratories for the characterization of physical surface properties of

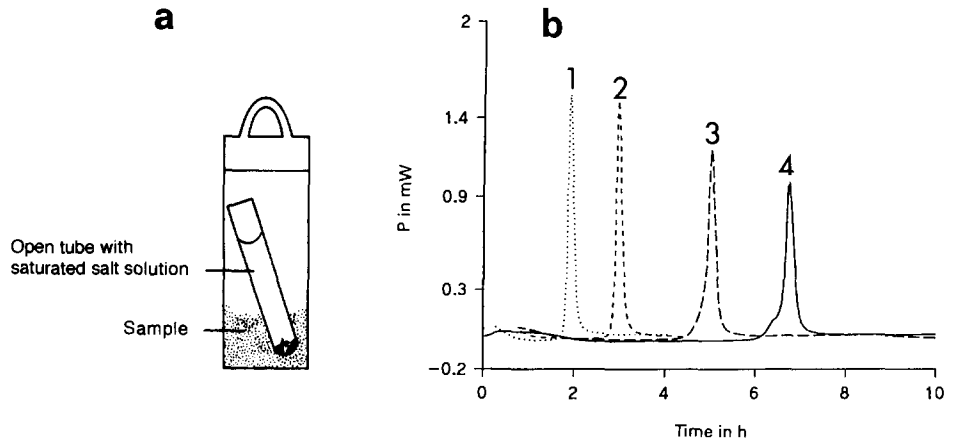

Fig. 2. Measurement of vapour sorption. (a) – Schematic picture of a simple experimental arrangement using a 'microhygrostat'. A microcalorimetric vessel is charged with a solid sample. An open tube with a saturated salt solution is placed in the vessel immediately before it is closed. The atmosphere above the salt solution has a well- defined vapour pressure (relative humidity, RH), and vapour will gradually be adsorbed by the sample until equilibrium has been reached; and (b) – results from experiments conducted at 25°C with 20 mg of spray-dried lactose powder using different salt solutions: (1) 85% RH, (2) 75%, (3) 65% RH and (4) 53% RH. (Adapted from [16]). The sharp peaks are caused by crystallisation of the amorphous fraction of the lactose sample initiated by the sorption of water vapour.

drug substances, excipients and of their combinations in formulations. The physical state of a solid material (crystalline, or partly amorphous, polymorphic forms) may depend on a certain step of the manufacturing process and can be of major importance in the subsequent handling of the material (e.g. flow-ability of a powder) and for the properties of the products (stability and bio-availability). Fig. 2(a) shows a simple microcalorimetric vessel used for such studies according to an experimental procedure developed a few years ago by Byström [16] and Angberg [17]. Fig. 2(b) shows results from water vapour sorption by lactose, a commonly used excipient. In a more sophisticated vapour sorption vessel, developed by Bakri [18] and produced commercially by Thermometric, a flow of carrier gas saturated with vapour is mixed with dry gas to give a predetermined vapour concentration of the gas flow, which is brought in contact with the sorbent. Very recently, various 'double twin microcalorimeters' have been developed, by which the sorption enthalpy as well as the sorption isotherm can be obtained [19,20].

3.3.2. *Adsorption of solutes*

Enthalpy of sorption of solutes by solids are often measured by use of titration microcalorimeters. The calorimetric vessel is then (preferably) charged with a suspension of the solid material which is titrated with the solution. The solid material can also be in larger pieces or in the form of a film, but a small specific surface area of the sorbent will give a small calorimetric signal and a higher sensitivity of the calorimeter may be needed. It should be noted, however, that molar enthalpies of solute adsorption (which normally involve a solvent displacement process) often is very high. In fact, undesirable sorption processes involving adsorption of, e.g. ions, by the walls of a microcalorimetric vessel made of glass or metal, can cause large systematic errors [21].

One important area of solute sorption microcalorimetry is in the field of off-shore oil recovery techniques, where detergents are used to form oil emulsions. For this reason, it is of interest to characterize the thermodynamic properties of adsorption of detergent molecules to mineral particles (see e.g. [22]).

3.4. *Vaporization (sublimation) processes*

Very little development work and few application studies in vaporization/sublimation microcalorimetry has been reported in recent years. However, there is a strong need for sublimation enthalpy values, e.g. in

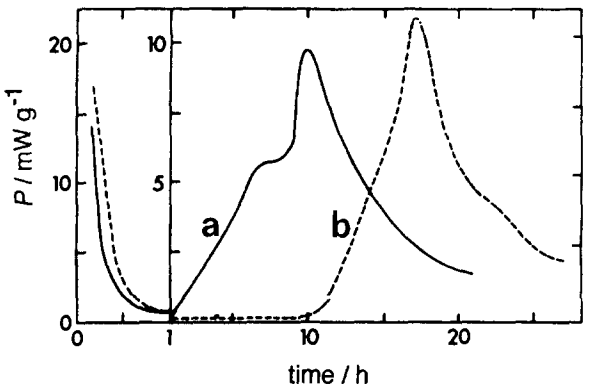

Fig. 3. Microcalorimetric records from hydration of cement. (a) – Normal hydration of a Portland cement; and (b) – retardation effect caused by addition of 0.4% Zn powder. (Adapted from [25]).

connection with studies of bio-thermodynamic model systems.

3.5. Monitoring degradation and curing processes

Many isothermal microcalorimeters are primarily used as monitors for slow degradation processes in technical materials, including characterization of incompatibilities between different materials. Important examples are the control of degradation processes in explosives and pharmaceutical products [23,24]. An early use of isothermal microcalorimetry was in investigations of different curing processes. As an example, power–time curves for cement hydration are shown in Fig. 3.

3.6. Living systems

Major systematic development work has been conducted in isothermal micro calorimetry, applicable to different kinds of living systems: microorganisms, human and animal cellular systems, small animals and materials from plants. Much of the work has been carried out with the aim of introducing the technique in applied areas, such as clinical analysis, pharmacology, ecology and agriculture. Many investigations have been reported from work conducted on the fundamental level and from 'demonstration experiments' in several applied areas, (see, e.g. [26–31]). As yet no important practical use has been established in work on living systems, but that time may soon come [32].

3.6.1. Microorganisms

Detailed reviews and discussions on calorimetry of growth and metabolism of microorganisms have been reported by Belaich [33] and James [34] for bacteria and by Lamprecht [35], Miles et al. [36] and Perry et al. [37] for yeast. In Gustafsson's [38] broad discussion on metabolism, both bacteria and yeast are treated. For references to work on mycoplasma, see [39]. Examples of mathematical modelling of microcalorimetric growth curves have been discussed by Takahashi and Yamano [40].

Much work has been conducted on investigating the effect of antibiotics on microorganisms (for reviews, see [27,34,36,41,42]. Fig. 4 shows a typical example from a study in that field which, at the time, was considered to be very promising for practical applications of isothermal microcalorimetry. Those expectations have not been realized. The same applied to techniques for identification of microorganisms by use of their very complex growth curves in complex media (see [34]).

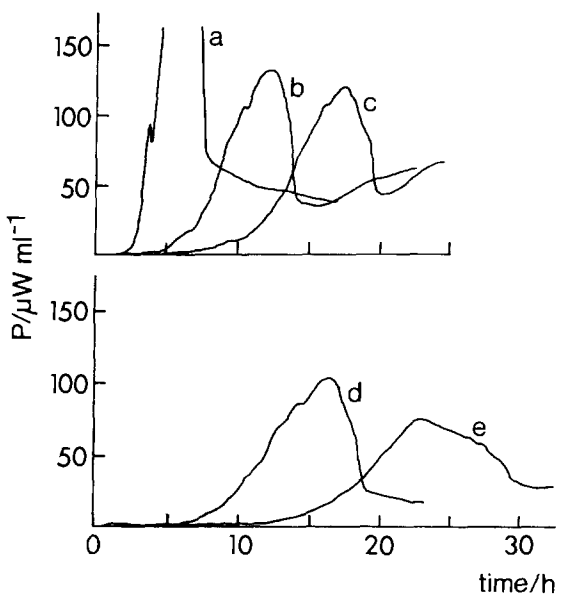

Fig. 4. Thermal power produced by *E. coli* cultured in the presence of tetracyclines (0.4 mg/ml) in trypticase soy broth. (Adapted from [43]). The antibiotic was added to the culture at time zero. (a) – Control experiment; (b) – tetracycline; (c) – doxycycline; (d) – oxytetracycline; and (e) –minocycline. Clearly, among the antibiotics tested, minocycline will depress growth of the bacteria for the longest time.

Following some early work on soil, sludge and waste water systems, it can be concluded that isothermal microcalorimetric techniques are now well developed for work of ecological interest on such systems [44]; possibly this application area will soon become of practical importance. Estimation of degradable ores and bacterial leaching using microcalorimetric techniques is an interesting application with some practical potential [45]. Ferry and Lovrien have developed sensitive microcalorimetric methods for determination of organic compounds in an aqueous solution [46], which may become of some practical importance, e.g. in monitoring low concentrations of certain pollutants in drinking water.

3.6.2. *Human and animal cells and tissues*

Developments of techniques for the measurement of thermal power from human and animal cells and tissues by isothermal microcalorimetry has made much progress during the past decade. Suitable instruments and working procedures have been developed and commercial instruments cover much of the needs for current research work in this field. Methodological work and reports on applications are well treated in review articles by Kemp [47–51] (see also his brief historical review of microcalorimetric work in this area [52]).

Many investigations for the purpose of clinical applications have been conducted on human cellular material, in particular by Wadsö [27] and Monti [53,54]. An interesting type of application, judged to be of immediate technical importance is illustrated by Fig. 5, showing the incompatibility between granulocytes (a type of white blood cells) and a membrane material [55].

Calorimetric characterization of interactions between drugs and cellular materials (cells, tissue pieces) from humans and animals is believed to be an area of significant potential [32], but the number of research groups currently reporting results from this area is not impressive. It is surprising, that the pharmaceutical industry has shown very little interest for developments in this area [9].

3.6.3. *Small animals*

Insects are usually easy to accommodate in a microcalorimetric vessel, but only a few investigations have been reported. Several studies have been conducted on small aquatic animals, to a large extent in connection with studies of anoxia [56]. Measurements on somewhat larger animals, like frogs and lizards, have been performed in 100 ml vessels of Calvet microcalorimeters (Setaram), (see e.g. [57]).

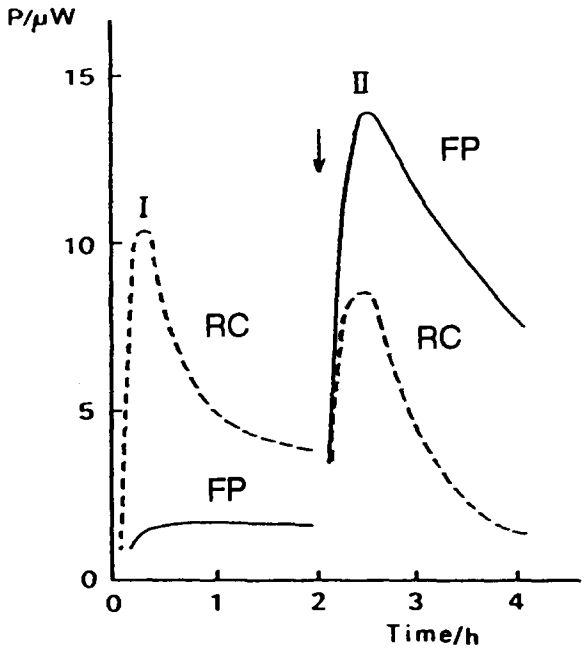

Fig. 5. Power–time curves from experiments with human granulocytes in contact with membranes made from fluorinated polypropylene (FP, a refence material) and regenerated cellulose (RC). (Adapted from [55]). At the start of the first part of the experiments (A) the cells were in the resting state. During the second part (B) the cells were stimulated by addition of zymosan added at the time indicated by the arrow. It is seen that during phase (A) the cells in contact with the reference polymer showed a low steady state heat production rate, whereas the larger and variable heat effect seen for the RC-curve indicates interaction between cells and the cellulose material. The adverse nature of that interaction is shown by results in the second part of the experiment (B) where the cells were activated by zymosan. A relatively weak heat burst was found for the cells in contact with the cellolose membrane, which is a sign of a decreased phagocytic response.

3.6.4. *Plant materials*

Calorimetric investigations on plant material have been concerned with the measurement of seed germination, cell cultures, tissue pieces and whole plants. Work in this field was initiated by Prat some 40 years ago [3]. Surprisingly, only a few scattered reports from this area appeared until a working party headed by

Criddle and Hansen, some 30 years later, initiated a very productive programme in plant calorimetry (for reviews and discussions, see [58–60]).

Most of the work reported has been on tissue pieces (shoots, leaves, stems, and roots). Many papers deal with the effect of stress factors like high or low temperature, high salt concentration, pollutants and the response to hormones and other metabolic effectors. There have been several reports by Criddle et al. [59] on correlations between heat production rate and biomass production, an area judged to have a significant practical potential. Practically no work has been reported on photo-microcalorimetry [60] applied on plant materials [61,62]. A gas perfusion microcalorimeter for studies of plant tissues [63] was recently redesigned to accommodate light guides [64].

4. Increasing the specificity of the measurements

Like all non-specific thermodynamic or analytical techniques, calorimetry has an advantage in its broad application range. Further, it is sometimes useful to employ completely nonspecific instrument techniques as monitors for complex and poorly known processes. It is thus more likely that unknown or unexpected processes will be recorded (discovered), compared to cases where specific analytical techniques are used. However, there are many cases, where the lack of specificity has disqualified the wider use of isothermal microcalorimetry as an analytical tool. It is therefore often desirable to combine isothermal microcalorimetry with specific analytical methods. Several methods have been explored in this connection, e.g. extracting small liquid or gaseous samples for analysis from a microcalorimetric batch vessel by means of a syringe needle [63], on-line analytical measurements in series with flow (perfusion) calorimeters, use of electrodes positioned in the microcalorimetric vessel [65] or the simultaneous measurements of thermal power and optical density [66]. Fig. 6 indicates schematically a technique for the measurement of thermal power from plant tissues under dark conditions, and the simultaneous measurement of carbon dioxide released and oxygen consumed in the process [67]. The method is based on the combination of results from two separate calorimetric measurements and measurement of the change of pressure in the closed system.

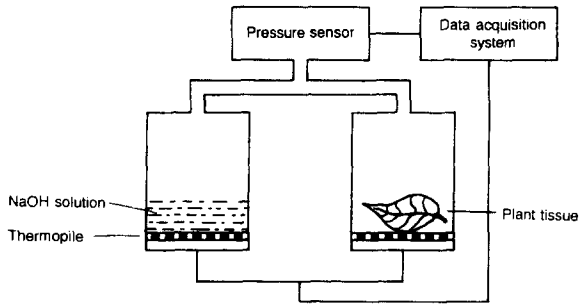

Fig. 6. Schematic picture of a double microcalorimetric assembly used for the simultaneous determination of thermal power, CO_2 and O_2 produced in experiments with plant tissues under dark conditions. (Adapted from [67]). A heat conduction differential scanning calorimeter from CSC (which has four calorimetric units) is used in the isothermal mode. The thermal power from the plant tissue is measured in one of the calorimetric units. CO_2 produced by the tissue will diffuse to the other calorimetric vessel, where it is trapped by NaOH solution. Its rate of absorption can be derived from the thermal power measured with that calorimeter. If the gas volume of the calorimetric system is known, results of pressure measurements will lead to values for changes of the oxygen concentration.

It is felt that further developments of combinations between isothermal microcalorimetric techniques and specific analytical methods are important for a wider use of isothermal microcalorimetry as a process monitor.

5. Are the results accurate?

Most calorimeters are calibrated by the release of electrical energy in an electrical heater. Such calibration procedures are convenient and are also very accurate from the point of view that electrical power or energy can be measured with an accuracy exceeding that of any calorimetric determination. However, sometimes the comparison between the heat released in the calibration heater and that evolved in the calorimetric experiment is not very close with respect to the heat flow patterns. The difference can significantly influence values for the fraction of the heat flow passing through the thermopile wall in a heat conduction calorimeter or the temperature signal(s) governing adiabatic conditions, or heat exchange corrections in adiabatic type calorimeters. Such problems can be specially important when considering isothermal

microcalorimeters where the mechanical design sometimes, from a calorimetric point of view, has been made far from ideal in order to allow certain processes to be conducted under well-defined chemical (physical and biological) conditions. It is then important to have available suitable test processes by which electrical calibration values can be checked. For some isothermal microcalorimeters electrical calibration should be avoided and replaced by standardized chemical processes (including dissolution and dilution processes). Several test and calibration processes suitable for different types of experiments have been proposed [68,69], but as yet there are no international recommendations in this area.

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