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ISOTHERMAL MICROCALORIMETRY
Current problems and prospects

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Abstract

A brief survey is given of recent developments and current activities in isothermal microcalorimetry. The discussion focuses on new methods in areas where the techniques have proved to be particularly useful or are promising to be so, in a near perspective. Some problems and limitations with current methods are also discussed.

Keywords: developments and applications, isothermal microcalorimetry, specific analysis

Introduction

The term 'isothermal microcalorimeter' is defined here as a calorimeter designed for essentially isothermal measurements, in the microwatt range. No distinction will be made between such instruments and 'nanocalorimeters', a term sometimes used for isothermal calorimeters where the power detection limit approaches a few nanowatt.

Different measurement principles are employed and practical designs vary depending on what type of experiment(s) the instruments are intended for. From the point of view of heat measurement principles one may divide isothermal microcalorimeters into three main groups: adiabatic, heat conduction and power compensation calorimeters [1].

In an ideal adiabatic calorimeter no heat exchange takes place between the calorimetric vessel and its surroundings. The amount of heat that is evolved or absorbed in an ideal adiabatic calorimeter is equal to the product of the measured temperature change and the heat capacity of the vessel, including its content. In practice semi-adiabatic calorimeters are often used and in accurate work it is then necessary to correct for the heat exchange with the surroundings.

In a thermopile heat conduction calorimeter heat released (absorbed) in the reaction vessel is allowed to flow to (from) a surrounding 'heat sink,' usually consisting of a metal block. A thermopile, positioned between the vessel and the heat sink, serves as a sensor for the heat flow. The total heat flow between vessel and the heat sink is proportional to the temperature gradient over the thermopile and thus to the measured thermopile potential. Heat flow sensors usually consist of thermopiles

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made from semi-conducting materials ('Peltier effect plates'). The time constants for the instruments are quite high, typically in the order of a few minutes. Much smaller time constants are obtained with miniaturised thermocouple plates in the form of 'chips', which recently have come into use [2]. However, such instruments are less sensitive than those employing larger size Peltier effect plates for which the surface area typically is a few cm^2.

In a power compensation calorimeter the thermal power from an exothermic process is balanced by a known cooling power (usually Peltier effect cooling), alternatively by a decrease of heating power. Endothermic processes are balanced by a known thermal power released in a heater or by reversing the Peltier effect current.

The first modern isothermal microcalorimeter was designed by Calvet in Marseille, and was based on Tian's thermopile heat conduction calorimeter [3]. The instrument was used by Calvet and his co-workers in many pioneering investigations of technical and biological interest [3] and became commercially available in the early sixties through Setaram, Caluire, France. About the same time, Benzinger, USA, reported another interesting thermopile heat conduction microcalorimeter [4], (for a short period produced by Beckman Instruments, USA). In particular the Calvet microcalorimeter, but also the Benzinger instrument, have had a very marked influence on the design of subsequently reported isothermal microcalorimeters.

In addition to the Setaram's microcalorimeters, the modular instruments marketed by CSC (Provo, UT, USA) and Thermometric (Järfälla, Sweden) are also of the thermopile heat conduction type. The principle used in Microcal's titration microcalorimeter (Northhampton, MA, USA) is different. In this instrument the temperature is allowed to increase during an experiment (as in a power compensation DSC, but the calorimeter may still be considered as 'isothermal' as the temperature increase is very slow.

In contrast to DSC, isothermal microcalorimeters form a very heterogeneous group with respect to their practical designs, in particular regarding the reaction vessels. Normally, isothermal microcalorimeters are designed as twin (differential) instruments. During the past two decades most development in isothermal microcalorimetry has been concerned with the design of new or modified reaction vessels for measurements at ambient temperature, with the focus on studies of aqueous solutions and biological systems [5, 6]. During the 1990s, methods for investigation of processes in the solid state became increasingly important, largely due to the interest in isothermal microcalorimetric techniques in the industry, in particular the pharmaceutical industry.

All isothermal microcalorimeters are thermodynamic instruments and can thus be used for determination of enthalpy changes. Measurements at different temperatures will lead to corresponding changes in heat capacity. Titration calorimetric methods can lead to a simultaneous determination of equilibrium constants and enthalpy changes, from which the changes in standard Gibbs energies and entropies can be derived.

The thermal power (heat production rate, dQ/dt) for a process is a thermodynamic as well as a kinetic property. In principle, all isothermal microcalorimeters can

therefore be used as kinetic instruments. Thermopile heat conduction microcalorimeters are especially useful for kinetic investigations of very slow processes. Special types of semi-adiabatic microcalorimeters, where the time resolution is about one millisecond, have been reported [6]. In no case calorimetric techniques are suitable for very fast kinetics.

Some important application areas

Isothermal microcalorimeters are used in thermodynamic and kinetic investigations and as general analytical tools, e.g. as 'process monitors.' The technique is applied in a wide range of fundamental studies and in technical applications in physics, chemistry and biology. In this section different experimental methods and some important applications will be discussed. The intention is not to present a review, but to focus on recent methodological progress and on the strength and the limitations of the technique.

Special attention is given to present and potential applications where isothermal microcalorimetry is primarily used as an analytical tool. Calorimetry is a non-specific technique and can thus, in principle, be applied to all types of systems and processes. This property makes calorimetry a particularly powerful method for the discovery of unexpected or unknown processes or reaction steps, for example in biology and when complex systems in technical products are investigated. However, the negative consequence of non-specificity is that the instrument signal is not very helpful in the identification of the studied process. Consequently, signals from complex reaction systems can be very difficult to interpret if they are not supported by results from specific analytical measurements. It can therefore be attractive to incorporate analytical sensors in the calorimetric vessel or, in case of a flow calorimeter, in the flow-line. A marked progress has recently been made in developments aiming at more specific measurements in isothermal microcalorimetry (see below).

Dissolution processes

Microcalorimetric methods for the determination of ΔH and ΔC_p for dissolution processes, and thus for transfer processes, have played an important role for the progress of thermodynamics of solute–solvent interactions. In particular, aqueous solutions have been studied, with the focus on hydrophobic or partially hydrophobic solutes. Instruments and working procedures have been described for microcalorimeters for dissolution of gases, liquids and solids and many series of gaseous and liquid compounds have been investigated [6]. Surprisingly, reports on microcalorimetric work in the area of solute–solvent interactions have been scarce during recent years. This area certainly needs much more input from isothermal microcalorimetry.

In addition to the fundamental work, microcalorimetric dissolution studies have become of practical importance in the characterisation of polymorphic properties, especially in the pharmaceutical industry.

Titration microcalorimetry and ligand binding processes

The thermodynamic characterisation of ligand binding processes is currently one of the most important areas of isothermal microcalorimetry [1]. By means of titration experiments it is often possible to determine, simultaneously, enthalpy values and corresponding equilibrium constants. Ligand binding studies involving e.g. metal ions, macrocyclic compounds and biopolymers are important in fundamental solution chemistry and biochemistry and have also become of significant practical value in development involving 'structure based' drug design.

A certain 'binding model': A+B=C or A+2B=AB_2 etc. must be assumed and used as a starting point in the calculations. Values for equilibrium constant(s) and change(s) in enthalpy are evaluated by some minimisation procedure after which the change in standard Gibbs energy and entropy can be derived. The calorimetric results can give support to a certain model, but cannot prove it is correct. It is felt that, at least for binding models more complex than simple 1:1 processes it is necessary to get support for the model by results of specific, analytical methods.

The driving force in the association between e.g. protein and drug molecules are to a large extent due to changes in the solvation of the molecules, (cf. the discussion above on the importance of solution and vaporisation studies). Freire and co-workers, and others, have approached the problem of correlating thermodynamic and structural parameters by dividing the $\Delta G°$ term into several structure related parameters, where the large contributions from the enthalpy and the entropy terms and the heat capacity changes are accounted for, see e.g. [7]. The contributions are to a large extent determined by changes in the exposure to water by (parts) of the reacting molecules.

Sorption of gases/vapours and solutes on solid surfaces

During the past decade the measurement of enthalpy of sorption (adsorption, absorption) became an important application area for isothermal microcalorimetry, in particular in the pharmaceutical industry. Measurements of sorption of solutes on solid particles (preferably in suspension) and on fibres can also be conducted by use of titration microcalorimeters. One area of practical importance for such studies is in connection with oil recovery techniques, where it is important to characterise the binding of detergent molecules to mineral particles.

The sorption of water vapour by materials like pharmaceuticals, foodstuff and fibres will often lead to significant changes of the technical and functional properties of the material. Briggner *et al.* [8] and Angberg *et al.* [9] showed that new and valuable information can be obtained by very simple sorption experiments, where static ampoules are used as reaction vessels, Fig. 1A. An open tube containing a saturated salt solution is placed in the calorimetric ampoule, which is charged with a sample in the form of fine particles. The salt solution will give the atmosphere in the reaction vessel a constant and well-defined relative humidity and water vapour will gradually be adsorbed on the sample until equilibrium is reached. The instrument will thus measure the sum of the endothermic enthalpy of vaporisation of water from the salt solu-

tion and the exothermic enthalpy of sorption of the vapour on the sample. In a more sophisticated method, Fig. 1B, a flow of carrier gas (with a constant or variable vapour composition) will pass through a perfusion vessel in the calorimeter [10]. Such measurements will lead to well-defined enthalpies of sorption.

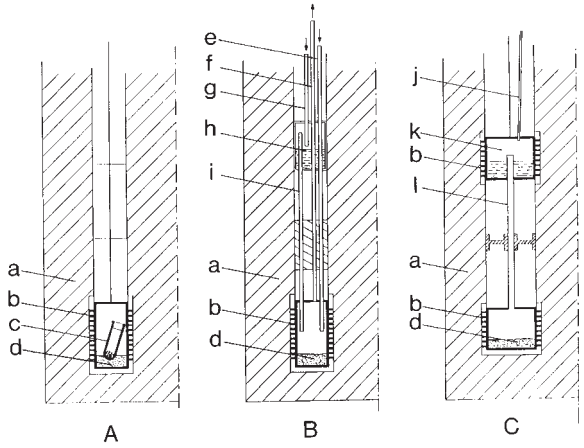

Fig. 1 Schematic pictures of three isothermal sorption microcalorimeters. The pictures do not show the reference parts of the twin thermopile heat conduction microcalorimeters
A – 'miniature humidity chamber technique'. a – heat sink; b – thermocouple plate; c – tube with saturated salt solution; d – sorption vessel with sample;
B – perfusion of vapour with controlled relative humidity. a, b and d as in A. e – tube introducing dry gas to sorption vessel; f – exit tube; g – tube introducing dry gas to humidifier chamber (h); i – tube carrying gas saturated with vapour to the sorption vessel, where it is mixed with dry gas from tube (e)
C – 'double calorimeter' for the simultaneous determination of sorption isotherms and enthalpy of vaporisation. a, b and d as in A. j – liquid injection tube; k – vaporisation calorimeter; l – diffusion tube

More recently, techniques using (twin) double microcalorimeters have been reported, by which both the sorption isotherm and the enthalpy of sorption can be obtained, Fig. 1C [11, 12]. Vapour formed in the upper vaporisation vessel will diffuse to the lower vessel containing the sample. From the measured enthalpy of vaporisation as a function of time the vapour flow rate is obtained. Using that value and known data for the vapour diffusion coefficient and the dimension of the tube connecting the two calorimeters, it is possible to calculate the sorption isotherm, assuming that near equilibrium conditions will exist in the sorption vessel.

Assessment of instability and non-compatibility

A significant part of the isothermal microcalorimeters that currently are used in industrial laboratories are employed for the assessment of non-stability of compounds and technical products. For investigations in this field it is usually important to use in-

struments with a very low limit of detection and good baseline stability (e.g. 0.1 μW during 24 h). The thermal power often appears as constant and the process may then be characterised by a single value for the thermal power. However, a thermal power value by itself does not give any information about the degradation rate, expressed in e.g. % of (active) material degraded per year. To obtain such information the enthalpy change (expressed in units of J mol^{-1} or J g^{-1}) must be estimated. Experiments in this field should therefore be supported by results of specific analysis as well as a thermochemical discussion.

The non-compatibility between two or more components of a product is an important technical problem, for which a microcalorimetric analysis often seems ideal. Each component is measured separately and from the results a thermal power value for the product is calculated, which is compared with corresponding experimental value. Closed ampoules are normally used as reaction vessels, but in some cases it is of interest to conduct the measurements using perfusion vessels with a flow of air or inert gas.

Willson, Beezer and their co-workers have reported methods for a detailed analysis of thermal power curves for very slow degradation processes. Values for the molar enthalpy change, rate constants and reaction orders can be derived [13, 14]. The authors showed that useful kinetic data can be derived for processes where the degradation rate is less than 1% per year.

Curing processes: cement, polymers

Cement hydration and polymerisation processes produce very high thermal powers. To monitor such processes it is therefore hardly motivated to use highly sensitive instruments. Still, cement industry has been using isothermal microcalorimeters in development and as a control instrument for a long time. This is due to the fact that thermopile heat conduction microcalorimeters are usually well suited for measurements of thermal powers, which are several orders of magnitude higher than their detection limit. However, in order to monitor post-curing processes (e.g. several months after a cement has hardened) and to follow the physical and chemical ageing of the products, a high sensitivity is needed.

Living systems

A large part of the development in modern isothermal microcalorimetry has been connected with needs in studies of living systems: microorganisms, animal (including human) cells and tissues, materials from plants and on small animals [6, 16]. In most cases such development has been motivated by potential uses of the method in the applied areas, but no application of real practical importance has yet been established. It is assessed that this is partly due to the fact that the sample throughput in a microcalorimeter is low compared to many other techniques. For example, cell-drug screening experiments in pharmacology or measurements of samples from large numbers of patients are hardly feasible with instruments currently available. Another

property that presently limits the practical use of isothermal microcalorimetry as a general analytical tool in applied biology, is the lack of specificity in the measurements and thus the difficulty to identify the events that are recorded, cf. below.

Microorganisms

In addition to fundamental studies much work on the effect of drugs on bacteria and yeast have been conducted, primarily to investigate the possible practical use in areas like clinical analysis, pharmacology and in food sciences. Many studies on mixed microbial systems, e.g. in soil, have also been conducted.

Animal cells and tissues

Much methodological progress was made during the past decade in work on animal cells and tissues. It is now possible to conduct measurements under well-defined and physiologically satisfactory conditions on cells in suspension, cells adhering to a solid surface and on tissue pieces (e.g. biopsies) [5, 6, 15, 16].

Significant fundamental work in physiology has been conducted. Many studies have been reported about work on different fractions of human blood cells, fat cells and on muscle biopsies from healthy subjects and from patients. Several studies have also been conducted on tumour cells and on cultured tissue cells. Investigations on human cellular systems have in most cases been made to explore the possible use of isothermal microcalorimetric methods in clinical analysis. It can be concluded that the non-specific calorimetric technique by itself is not very useful as a diagnostic tool in medicine. However, the potential as a prognostic tool is more promising [16].

Materials from plants

Surprisingly, after the pioneering work of Calvet and Prat on plant materials about 50 years ago [3], only scattered reports appeared until quite recently. Hansen, Criddle and their co-workers have reported significant method development and investigations in several areas of practical importance [17]. Most work has been conducted on tissue pieces such as leaves, stems and roots and has often been concerned with the effects of stress factors, like high salt concentration, pollutants and high or low temperature.

Practically all measurements on plant material have so far been made in the dark, but it was recently shown that photo-calorimetric measurements on plant tissue can be made using a regular isothermal microcalorimeter equipped with some attachments [18].

Towards more specific measurements

The thermal power–time curve recorded for a complex reaction system represents the sum of all part-processes, except for possible contributions of reactions with zero enthalpy change. It was pointed out earlier that such detailed, but non-specific, re-

cords are often difficult to interpret. Calorimetric records are more informative if they are extensively supported by results of specific analyses, if possible expressed in molecular terms. Further, where possible, analytical measurements should be conducted continuously and on the reaction system that is measured with the calorimeter. In flow calorimetry it is usually possible to make analytical measurements 'on line', or on samples extracted from the flow line. In microcalorimetric experiments conducted batch-wise it may be possible to extract samples from the calorimetric vessel, but such operations will often disturb calorimetric measurements that are conducted on the microwatt level. The combination of batch-microcalorimetric experiments with continuous analytical measurements is often best conducted using analytical sensors, e.g. electrodes, positioned in the calorimetric vessel. In several cases such combined microcalorimetric-analytical techniques have recently been explored. Figure 2 shows schematically a microcalorimetric vessel fitted with electrodes and also serving as a spectrophotometric cell.

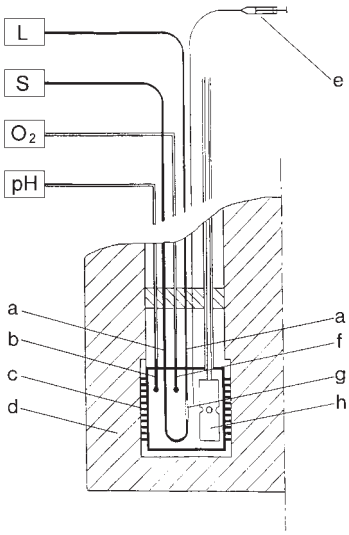

Fig. 2 Schematic picture of a thermopile heat conduction microcalorimeter equipped with electrodes and spectrophotometer [19] (the reference part of the twin calorimeter is not shown). L – light source; S – monochromator and diod array detector; O_2 – electronic unit for the polarografic oxygen electrode; pH – pH meter; a – quartz rod; b – pH electrode; c – thermocouple plate; d – heat sink; e – syringe and syringe drive; f – oxygen electrode; g – gap between the quartz rods; h – 'turbine stirrer'

In Fig. 3 the results obtained by use of that vessel in an experiment with *E. coli* grown on complex medium are shown. The sample compartment was completely filled with medium. The apparent optical density ('turbidity'), A^{ϕ}, can be assumed to be proportional to the cell concentration. It is seen that all oxygen has been consumed soon after 3 h, curve b, after which the power–time curve will show a second peak. It

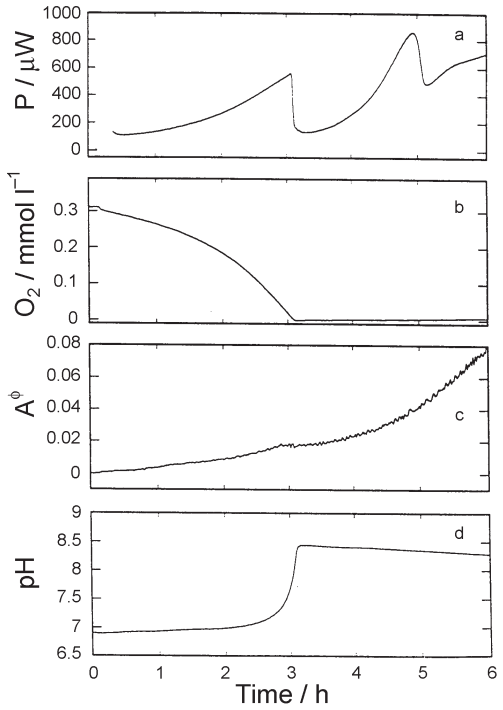

Fig. 3 Records from a growth experiment with *E. coli* in complex medium, using the microcalorimeter shown in Fig. 2 [19]. The sample compartment was completely filled with medium. a – thermal power *vs.* time. The experimental curve was dynamicaly corrected; b – oxygen concentration *vs.* time; c – apparent optical density (turbidity), A^{ϕ} – *vs.* time, d – pH *vs.* time

is seen that the peak in the anaerobic part of the thermal power–time curve is not significantly reflected in the turbidity curve. The results suggest that the calorimetric curve accounts for aerobic and anaerobic growth phases and, in addition, for one or more processes that do not lead to biomass production. From the shape of the aerobic parts of curve a, b and c and for the anaerobic part of curve d, the generation time for the bacteria during the two growth processes can be derived. Clearly, for complex processes of this kind, it would be desirable to use additional specific sensors. A wide range of sensors are available, which may be incorporated into microcalorimetric vessels, without any significant interference with calorimetric measurements, even if they are conducted in the microwatt range.

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