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RECENT DEVELOPMENTS IN REACTION AND SOLUTION MICROCALORIMETRY *

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ABSTRACT

Recent developments in microcalorimetric methods for measurements of processes in solution, dissolution of gases, liquids and solids and in work on living cells have been reviewed. The discussion is limited to isothermal techniques which are used in the ambient temperature range and at atmospheric pressure.

INTRODUCTION

The term "microcalorimeter" is by no means well defined. In this report the micro prefix is primarily used for calorimeters for which the practically useful sensitivity is about 1 μW or better.

Recent developments in microcalorimetry have been very substantial and easy-to-use techniques which are now available for a wide range of experimental conditions. This review will concentrate on the developments in experimental techniques which have taken place since 1980 and are of a particular interest in studies of processes in solution, for dissolution processes involving gases, liquids and solids and in work on living cells. The discussions are limited to isothermal techniques which are used in the ambient temperature range and at atmospheric pressure.

SOME COMMENTS ON INSTRUMENT DESIGN

Sensitivity

Hansen and Eatough [1] analyzed the sensitivity limits for microcalorimeter designs relevant to application in the areas discussed here. They con-

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cluded that, for short-term experiments, adiabatic or semi-adiabatic (isoperibol) flow calorimeters are the most sensitive instruments in terms of minimum detectable heat quantities. But in current practice it is undoubtedly heat conduction calorimeters of the batch type, including instruments used in stepwise titrations, which are most common when very high sensitivity is needed.

Today, and for the past few decades, the need for increased sensitivity in biocalorimetry is probably the most important driving force for progress in isothermal as well as in temperature scanning microcalorimetry. In fact, most instruments referred to in this paper were primarily designed for work in biochemistry and on living cellular systems. In biochemical work, e.g. in ligand binding studies, there is definitely still a need for more sensitive calorimeters, primarily because they would make it possible to work with smaller amounts of very expensive compounds. Many interesting processes, involving very slightly soluble compounds, could be measured if more sensitive instruments were available. Sometimes work on living cells would also gain from more sensitive calorimeters. But it is not only higher instrument sensitivities that are called for. We also need developments of measurement procedures which will allow accurate work with increasingly smaller samples.

Stability

A long term "baseline stability" of the microcalorimeter is needed in many applications, e.g. in slow chemical reactions, in the dissolution of slightly soluble compounds using flow vessels, and in many types of investigation involving living cells. Today, well-designed twin microcalorimeters of the heat conduction type show long-term stabilities as good as ± 50 nW over 24 h [2]. For the temperature range 5–90°C, thermostated water baths still seem to be the best for providing the necessary constant temperature environment. Placed in an ordinary laboratory room, with temperature fluctuations not larger than ± 1 K, comparatively simple designs can be stable to within $\pm 1 \times 10^{-4}$ K over several days [2].

It should be noted that, in many types of work conducted on a very high sensitivity level, one can often accept a significant, but smooth, baseline drift. For instance, this is the case in stepwise titration (ligand binding) calorimetry, provided that the binding process is fast and that the time constant of the instrument is small in comparison with the baseline irregularities.

Time constant

In recent years, significant progress has been made in the use of microcalorimetric techniques in the determination of rather fast reaction rates.

Such instruments now have a time resolution of a few ms. This is not much faster than obtained with Roughton's [3] instrument some 40 years ago, but it is obtained with very much smaller amounts of substance. Usually the limiting factor for these instruments is the disturbances caused by the necessary forceful mixing procedure. The sensitivities are in many cases on a typical microcalorimetric level, whereas the long-term stability is usually low. In most cases semi-adiabatic instruments are used with a thermistor or a thermopile as the thermal sensor.

Modes of operation

Stepwise batch titration microcalorimetry has developed significantly during this decade, in particular with respect to the decreased amount of material needed and to the speed at which the measurements can be performed [4–8]. This also applies to stopped-flow calorimeters primarily used in kinetic measurements [9–13]. Continuous-flow mixing microcalorimetry is frequently used in ligand binding studies and in the determination of mixing and dilution enthalpies, and flow-through methods are of significant importance in work on suspensions of living cells. Surprisingly, recent instrument developments in these areas seem quite marginal. More progress can be noted in (biological) perfusion calorimetry [6,14,15] where the material, e.g. pieces of tissue or cells adsorbed on microcarriers, is retained in the calorimetric vessel while the medium is passing through.

A problem common to all techniques of continuous-flow calorimetry, and frequently a limiting factor, is the difficulty of maintaining a constant and pulse-free flow. For aqueous systems peristaltic pumps are dominating, but their flow performances have hardly been improved during the last two decades. For some applications, such as in the mixing of flows, high-quality HPLC pumps have proved to be very useful [16].

Techniques used for stirring and mixing are often critical factors in microcalorimetry. Rotation or rocking of the whole microcalorimeter with liquids or suspensions contained in open dual compartments is very efficient but the heat of friction is usually large and its variation may be a limiting factor for the instrument. In titration calorimeters and in several novel dissolution calorimeters one of the reaction components is injected into the calorimeter vessel by use of a syringe fitted with a very thin delivery tube. Procedures to account for diffusion and leakage problems have been proposed [17]. For batch reaction vessels having about the same width and length, the rotation of a conventional propeller normally serves well as a mixer, but for tall and comparatively narrow vessels, "turbine" stirrers [6,18] have recently proved to be more efficient. With these stirrers the liquid is circulated both horizontally and vertically and can often also be used for suspensions, e.g. with cells attached to microcarriers. For such systems it is possible to perfuse medium through the vessel without loosing any particles

[18]. A disc oscillating vertically can also be a very efficient stirrer in work with suspensions. The thermal effect accompanying continuous stirring in a small microcalorimetric vessel is now often kept at a level of ≤ 1 μW and will usually not cause any measurement problem.

Living cells

In calorimetric work on cellular systems, the need of adequate and well-defined physiological conditions must be observed, but there are many examples in recent literature where this is not the case. Among the factors often causing problems are: supply of oxygen (without disturbing the calorimetric signal), changes in pH, sedimentation, uncontrolled adhesion of cells and changes in viscosity of cell suspensions, causing changes in the heat of stirring. Very significant progress with respect to such problems has been made during the last two years [19].

A note about accuracy

Practically all processes are accompanied by heat effects. This makes reaction calorimeters suitable as general process monitors in addition to their use as thermodynamic instruments but it will also make all kinds of calorimeters very vulnerable to systematic errors. Such risks tend to be larger the smaller the investigated heat effects. Further, in many microcalorimeters the vessels must be a compromise between factors important for a good calorimetric design and those necessary for a certain measurement function [19]. In the author's opinion, these facts are frequently overlooked, in particular when commercial instruments are used. Different types of test reactions should be used much more frequently. In many cases it is, in fact, suitable to calibrate the instrument by use of a chemical reaction rather than using electrical heating. Work on such problems has been conducted in our laboratory for some time [20]. For microcalorimetric flow-through and perfusion vessels, calibration problems can be particularly difficult. In such cases reaction mixtures where triacetin is hydrolyzed in different imidazoleacetate buffers have been found useful [20,21]. The thermal power values for mixtures investigated so far are in the range 7–100 μW g^{-1} (25 or 37°C). Power values which are almost constant or slowly decreasing can be accurately predicted ($\pm 0.5\%$) over a time period of ≥ 20 h.

SOME MICROCALORIMETRIC DESIGNS

Here follow brief comments on some instruments which may serve as examples of the significant progress made in the field treated in this report.

Reaction and titration microcalorimeters

Gill and co-workers have reported several interesting design features from their work on very sensitive and fast titration calorimeters. Their latest design [5] is a stirred twin heat conduction calorimeter using a feed back principle which reduces the equilibration time. The reaction volume is only 250 μl and the sensitivity was reported to be about 5 μJ. Glass reaction vessels are moulded with a low melting alloy into a brass cup in contact with the thermopiles. Injections are made by use of a rotating syringe mounted above the vessel. The injection needle which reaches into the centre of the vessel has a shape which makes it also serve as a stirrer. The vessel is kept completely filled and thus a small part of the contents is forced out of the vessel at each injection. This requires the use of a dilution equation when the results are calculated [4]. The thermopile signal is employed for the regulation of an electrical heating current. The heat quantity determined is thus the sum of the thermopile potential–time integral and the decrease of the electrical (heating) power integral. By use of this feed back principle the main period for a fast reaction is reduced from 10 to 3 min.

In the author's laboratory a system of microcalorimeters with several different functions has been developed, and is made commercially available by ThermoMetric (LKB) Järfälla, Sweden. The basic unit [2] consists of a thermostated water bath ($\pm 1 \times 10^{-4}$ K) in which up to 4 microcalorimeters can be placed and independently operated. Normally, twin heat conduction calorimeters equipped with semiconducting thermopiles are used. The calorimetric vessels are designed to form a modular system. A flow-mixing vessel and a flow-through vessel can be wound on the outside of the tube-shaped vessel holders, where different cylindrical insertion vessels can be introduced. The series of insertion vessels presently include simple sealed ampoules and several mechanically similar but functionally quite different vessels for titration [6] and for biological perfusion experiments [6,14], for dissolution of solids [22], liquids [23] and gases [24], for adsorption of solutes on solids [25] and for measurements of light-induced processes [26]. When simple ampoules are used, the baseline stability is about ± 50 nW per 24 h. For the flow vessels and for the more complex insertion vessels the stability is slightly lower. Thorén et al. [27] have analyzed in considerable detail disturbances caused by the introduction of a simple insertion vessel into this type of instrument. With the flow-mixing version and employing a novel "closed circuit" method, Sari et al. [28] determined the binding constant, enthalpy change and stoichiometry values of a protein–ligand complex using only 2 ml of 10^{-5} M protein solution.

In several studies Freire and co-workers [29] have reported the use of a very sensitive and fast twin titration calorimeter where parts of the basic units of the ThermoMetric microcalorimetric system are used. The instru-

ment appears to be influenced by the Gill titration calorimeter [5] but details of the design do not seem to be reported.

Brandts and co-workers have described a very sensitive adiabatic microtitration calorimeter [8] which is commercially available through Micro Cal, Inc. (Northhampton, MA, U.S.A.). The stirring–injection technique is similar to that used in Gill's instrument [5]. Reaction vessels, volume 1.4 ml, are permanently mounted on either side of a semiconducting thermopile and the half-time response is only about 7 s. With the type of adiabatic compensation used, the temperature will increase continuously at about 1 K day^{-1}.

Mudd et al. [30] reported a typical twin heat conduction calorimeter and analyzed its performance in considerable detail. The reagents are mixed by rotating the calorimeter block where each vessel has two open compartments. The vessels, made from thin Kel F, can be taken out of their holders. Some characteristics for this instrument are as follows: solution volume 0.3 ml; baseline stability 0.2 μW over 48 h; thermal power resolution 0.2 μW; signal rise time 122 s (10–90% response). This instrument was partly designed for use as a kinetic instrument for reactions with half-lives in the range of 0.1–600 s. It was shown, however, that small variations in the air gap between vessel and vessel holder seriously affect the kinetic measurements. In a very recent paper, Berger et al. [11] report the use of the same calorimeter with a polypropylene mixing cell for fast stopped-flow measurements. Reagent volumes are in the range of 25–100 μl and the sensitivity is about 10 μJ.

Mudd and Berger [10] have described a stopped-flow calorimeter for which excellent performance data were reported. The instrument, a twin calorimeter using the thermopile heat conduction principle, is designed for enthalpy measurements but also for moderately fast enzyme kinetic studies (see below). Semiconducting thermopiles are placed along a 80 mm reaction tube made from tantalum. Typically, 80 μl of each reagent is injected. Mixing time is 0.6 s and the re-equilibration time is 150 s. The baseline stability was reported to be 0.1 μW over a 4 h period. Reaction heats as small as 20 μJ can be measured with a precision of 15%.

Instruments for studies of fast reaction rates

Several microcalorimeters have been developed in a direction which will make them useful for kinetic characterization of rather fast reactions, e.g. enzymatic processes. In particular, Berger and co-workers have been active in this area [9,11,30]. Balko et al. [9] reported the design of a fast stopped-flow calorimeter using a thermistor as temperature sensor in a semi-adiabatic instrument. At a sensitivity of 1×10^{-4} K, the time resolution was reported to be 3 ms.

The twin titration calorimeter reported by Kodama and Woledge [7] has a volume of 1–2 ml and a rather modest sensitivity of 0.2 mJ. But the

equilibration time following a fast process is only 1–2 s and the calorimeter is therefore useful for many types of kinetic measurements. This instrument uses a 30 junction thermopile made from thin electroplated Cu–constantan wire with low heat capacity and low thermal conductivity. The instrument can be characterized as a semi-adiabatic calorimeter. Stirring is achieved by use of a teflon disc which oscillates vertically.

In a recent paper, Howarth et al. [13] report the design and properties for a stopped-flow microcalorimeter designed specifically for enzyme kinetic measurements. It is a twin calorimeter of the semi-adiabatic type which is given a configuration similar to optical stopped-flow instruments. The thermal detector consists of a 100 junction constantan–chromel thermopile which is arranged between a thin-walled, tube-shaped reaction vessel made from epoxy resin and a surrounding water bath (not actively thermostatted during the measurements). This instrument has a time resolution of only a few ms but its temperature sensitivity is low, in the range of 1 mK (it is hardly a microcalorimeter according to the definition used in this paper). As the authors remark, further development of this system should be possible.

Dissolution calorimeters

An interest for thermodynamic properties of hydrophobic groups in aqueous solution and its consequences for the properties of many biological systems, "hydrophobic interaction" has initiated intense development work by Gill and Wadsö and their co-workers on new designs of dissolution calorimeters. In all cases microcalorimetric flow vessels were used with twin thermopile heat conduction calorimeters.

Gill and Wadsö reported the design of two different vessels for the dissolution of slightly soluble gases in water, e.g. hydrocarbons and the rare gases [31]. In a batch method, about 0.4 ml or less of an accurately known amount of gas is injected into the solvent flow and the resulting dissolution heat is determined. In a steady-state method, a very slow flow of gas is continuously supplied to the flow vessel where a steady-state situation will be established. In this method, the molar enthalpy of solution is calculated from the values of the gas flow and the measured steady-state value for the thermal power. The first versions were followed by improved designs: a steady state method, using a larger surface area for the contact between gas and solvent [32] and a batch method employing a vessel where the gas bubble and the solvent flow is stirred [24]. The vessel is very similar to our titration vessel [6] and is thus part of our "modular vessel system" (see above).

A method for the dissolution of hydrophobic liquids in water was reported several years ago [33]. It is based on the principle that a small quantity of liquid, about 3 μl, is injected into a flow of water in a calorimeter vessel made of a teflon tube formed into a spiral. The hydro-

phobic solute will be smeared out on the inside of the hydrophobic tube, thus forming a large surface area which will optimize the dissolution process. This instrument was subsequently redesigned and different types of coil material have been used. The latest version [23] is part of the modular system of microcalorimetric vessels mentioned above. The vessel can be used for measurements of slightly as well as of easily soluble liquid solutes. For very slightly soluble solutes the measurement time may be up to 20 h, using a solvent flow rate of about 25 $cm^3\ h^{-1}$.

Another part of the modular system is a vessel for the dissolution of slightly soluble solids [22]. It is based on the principle that a small amount of solid material (a few mg) is charged into a perfusion vessel filled with saturated solution. After equilibration, the flow of pure solvent, and thus the dissolution process, starts. Murphy and Gill [34] have recently reported a different dissolution technique for slightly soluble solids in which they used the Gill titration calorimeter [5]. To a saturated solution with suspended fine particles of the solid, a small amount of solvent (water) is added. From values for the solubility of the material, the known amount of solvent injected, and the measured heat, the molar heat of dissolution is calculated. The authors point out that, by measuring the enthalpy of dissolution at several temperatures, it is in principle possible to use this method without an independently determined value for the solubility. It should be noted that a solid in contact with a saturated solution may be in a different thermodynamic state than a dry sample. In a very recent design from our laboratory, 4 samples of readily or slightly soluble solids, each about 1 mg, can be "injected" consecutively into a stirred 10 ml vessel [35]. The vessel can be operated in the batch or the perfusion mode.

Maria et al. [36] have described a vessel for the dissolution of readily soluble gases using a Tian–Calvet microcalorimeter. Several other dissolution vessels for rather small quantities of solids have been reported recently, notably by Krestov and co-workers (see, for example, refs. 37 and 38).

Instruments for measurements of living cells

Calorimeters suitable for measurements of different types of cell systems have recently been reviewed [19] and the discussion here will be very brief.

Oxygenation of fast-growing microbial cultures remain a difficult problem. Fujita and co-workers have reported the design of a rotating batch calorimeter equipped with vessels specially designed for studies of aerobic microbial growth. A recent version [39] can be used in the batch or the flow mode and is commercially available from Tokyo Rico Co., Tokyo. The instrument reported by Ishikawa et al. [40], is designed to meet the oxygenation problem by the use of very efficient stirring. This causes a significant thermal disturbance but high oxygen consumption is always accompanied by a high heat production rate (this type of instrument is not used on a

"microcalorimetric" level). Instead of vigorously agitating the gas–liquid system in aerobic microbial experiments, Dermoun et al. [41] chose to work with shallow reaction vessels in which the medium has a large contact area with the gas phase.

Yamamura et al. [42] have designed a twin stopped-flow microcalorimeter for work with animal cell systems. The instrument is commercially available from ESCO Ltd, Muashino City, Tokyo. Yamamoto and Aki [43] have reported a differential flow microcalorimeter with a power resolution of 0.5 μW. Mixing of reagents with cells can take place immediately before the flow cell. Multi-vessel instruments have been reported by Hammerstedt and Lovrien [44] and by Takahashi and co-workers [45] primarily for use in the serial analysis of cellular material.

The commercial microcalorimeter BIO-DSC [46], produced by Setaram (Caluire, France) can be used both in temperature scanning measurements and in isothermal batch or flow measurements, e.g. on living cells. The instrument employs the heat conduction principle and the sensitivity in isothermal measurements is reported to be 0.2 μW.

In the author's laboratory, microcalorimetric techniques for investigations of animal (human) cells have been much studied during recent years. In all cases, our titration–perfusion vessels (volume 1–3 ml) [6,14], have been used. It is now possible to keep cells in uniform suspension under conditions where the cells do not appear to be damaged, reagents can be added during the experiments and measurements can be made on cells adhering to different types of support while fresh medium is perfused through the vessel. Very recently, a vessel has been equipped with electrodes for the measurement of pH and oxygen concentration simultaneously with the calorimetric measurements [47].

REFERENCES

1 L.D. Hansen and D.J. Eatough, Thermochim. Acta, 70 (1983) 257.
2 J. Suurkuusk and I. Wadsö, Chem. Scr., 20 (1982) 155.
3 F.J.W. Roughton, Proc. R. Soc. London Ser. A, 104 (1930) 376.
4 R.B. Spokane and S.J. Gill, Rev. Sci. Instrum., 52 (1981) 1728.
5 I.R. McKinnon, L. Fall, A. Parody-Morreale and S.J. Gill, Anal. Biochem., 139 (1984) 134.
6 M.G. Nordmark, J. Laynez, A. Schön, J. Suurkuusk and I. Wadsö, J. Biochem. Biophys. Methods 10, (1984) 187.
7 T. Kodama and R.C. Woledge, Rev. Sci. Instrum., 56 (1988) 40.
8 T. Wieseman, S.. Williston, J.F. Brandts and L.-N. Lin, Anal. Biochem., 179 (1989) 131.
9 B. Balko, P. Bowen, R.L. Berger and K. Anderson, J. Biochem. Biophys. Methods, 4 (1981) 1.
10 C.M. Mudd and R.L. Berger, J. Biochem. Biophys. Methods, 17 (1988) 171.
11 R.L. Berger, C.P. Mudd, T. Clem, T. Kolobow, E. Beile, P.C. Simons, S. Michel, W. McClintock, J. Biochem. Biophys. Methods, 18 (1989) 113.

12 T. Kodama, Netsu Sokutei, 15 (1988) 2.
13 J.W. Howarth, N.C. Millar and H. Gutfreund, Biochem. J., 248 (1987) 677.
14 A. Schön and I. Wadsö, J. Biochem. Biophys. Methods, 13 (1986) 135.
15 B. Fagher, M. Monti and I. Wadsö, Clin. Sci., 70 (1986) 63.
16 D. Hallén, I. Wadsö, D.J. Wasserman, C.H. Robert and S.J. Gill, J. Phys. Chem., 92 (1988) 3623.
17 S.-O. Nilsson and I. Wadsö, J. Chem. Thermodyn., 16 (1984) 317.
18 A. Schön and I. Wadsö, Cytobios, 48 (1986) 195.
19 I. Wadsö, in A.M. James (Ed.), Thermal and Energetic Studies of Cellular Biology Systems, Wright, Bristol, 1987, p. 34.
20 L.-E. Briggner and I. Wadsö, J. Biochem. Biophys. Methods, in press.
21 A.-T. Chen and I. Wadsö, J. Biochem. Biophys. Methods, 6 (1982) 297.
22 S.-O. Nilsson and I. Wadsö, J. Chem. Termodyn. 18 (1986) 1125.
23 D. Hallén, S.-O. Nilsson and I. Wadsö, J. Chem. Thermodyn., 21 (1989) 529.
24 D. Hallén and I. Wadsö J. Chem. Thermodyn. 21 (1989) 519.
25 M. Carlsson, G. Olofsson and I. Wadsö, to be published.
26 C. Teixeira and I. Wadsö, J. Chem. Thermodyn., in press.
27 S.A. Thorén, J. Suurkuusk and B. Holma, J. Biochem. Biophys. Methods, 18 (1989) 149.
28 J.C. Sari, R. Gilli, V. Peyrot and C. Briand, Thermochim. Acta, 147 (1989) 119.
29 M. Myers, O.L. Mayorga, J. Emtage and E. Freire, Biochemistry, 26 (1987) 4309.
30 C. Mudd, R.L. Berger, H.P. Hopkins, W.S. Friauf and C. Gibson, J. Biochem. Biophys. Methods, 6 (1982) 179.
31 S.J. Gill and I. Wadsö, J. Chem. Thermodyn. 14 (1982) 905.
32 S.F. Dec and S.J. Gill, Rev. Sci. Instrum., 55 (1984) 765.
33 S.J. Gill, N.F. Nichols and I. Wadsö, J. Chem. Thermodynamics, 7 (1975) 175.
34 K.P. Murphy and S.J. Gill, Thermochim. Acta, 139 (1989) 279.
35 D. Hallén, E. Qvarnström and I. Wadsö, to be published.
36 P.-C. Maria, J.-F. Gal, L. Elegant and M. Azzaro, Thermochim. Acta, 115 (1987) 67.
37 G.A. Krestov, V.N. Prorokov and V.V. Dolotov, Zh. Fiz. Khim., 56 (1982) 238.
38 V.P. Korolev, D.V. Batov, V.N. Vandyshev and G.A. Krestov, Zh. Fiz. Khim., 57 (1983) 253.
39 K. Kobayashi, T. Fujita and S. Hagivara, Thermochim. Acta, 88 (1985) 329.
40 Y. Ishikawa, M. Shoda and H. Maruyama, Biotechnol. Bioeng., 23 (1981) 2629.
41 Z. Dermoun, R. Boussand, D. Cotton and J.-P. Belaich, Biotechnol. Bioeng., 27 (1985) 996.
42 M. Yamamura, H. Hayatsu and T. Miyamae, Biochem. Biophys. Res. Commun., 140 (1986) 414.
43 M. Yamamoto and H. Aki, J. Biochem. Biophys. Methods, 16 (1988) 271.
44 R.H. Hammerstedt and R.E. Lovrien, J. Exp. Zool., 228 (1983) 459.
45 T. Kawabata, H. Yamano and K. Takahashi, Agric. Biol. Chem., 47 (1983) 1281.
46 L. Benoist and F. Pithon, Thermochim. Acta, 72 (1984) 189.
47 P. Bäckman, A. Schön and I. Wadsö, to be published.