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SOME DEVELOPMENTS IN REACTION AND SOLUTION MICROCALORIMETRY, WITH PARTICULAR REFERENCE TO ITS USE IN BIOCHEMISTRY AND CELLULAR BIOLOGY *

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ABSTRACT

The field of isothermal microcalorimetry as applied to systems of interest for aqueous solution chemistry, biochemistry and in cellular biology is briefly reviewed. Some techniques recently developed in the author's laboratory are described.

INTRODUCTION

Calorimetric work of biological interest ranges from studies of water and its interactions with simple model substances to work on living systems. Some investigations can be characterized as thermodynamic, whereas others are of a more general analytical nature [1–3]. Measurements are performed under essentially isothermal conditions or as temperature-scanning experiments. The latter are obtained with differential scanning (micro)calorimeters (DSC) and are widely employed in studies of thermal transitions of lipids, proteins, nucleic acids and their aggregates. This paper, however, will deal with isothermal microcalorimetry only, first in general terms and then with regard to some areas where it is currently used in aqueous solution chemistry, biochemistry and cellular biology. In the second part of the paper, some microcalorimetric techniques recently developed in our laboratory for these areas will be described.

SOLUTION AND REACTION MICROCALORIMETRY

No well-defined borderline exists between "ordinary" calorimeters, or "macrocalorimeters", and "microcalorimeters". The micro- prefix normally indicates a sensitive instrument which can be used with small sample

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quantities. For micro-reaction and -solution calorimeters, the power sensitivity is typically of the order of 1 μW or better and sample volumes are a few millilitre or less. The amount of material reacting is typically on the micromolar level, corresponding to a heat evolution of the order of a few milli-Joules.

Modern microcalorimeters have dramatically expanded the range accessible for high-quality thermochemical work on expensive material, slightly soluble compounds, reactions involving high molecular weight compounds and in the characterization of living cellular systems. However, thermochemical work on the macro level often leads to more precise and more accurate results [1–4]. Naturally, systematic errors are often more difficult to cope with in microcalorimetry than when comparatively large heat quantities are involved. Reaction and solution calorimeters used on the macro level are often isoperibol calorimeters, or of the adiabatic shield type. They are practically always single-vessel calorimeters. The corresponding microcalorimeters are most commonly twin instruments of the thermopile heat-conduction type. Their good baseline stabilities often make them suitable for studies of very slow processes, e.g., in dissolution of slightly soluble compounds and in living cell experiments. When two or more components are brought together in a calorimetric experiment, it is usually done by means of an ampoule or burette technique, or by mixing the contents of two compartments. These techniques are known as batch procedures. A continuous-flow or injection technique can also be used. Procedures of this kind can be very convenient and are often used in microcalorimetry.

With batch procedures, the method chosen for initiation of the process is very critical. Methods frequently used in macrocalorimetry, e.g., breaking of a glass ampoule or opening of a valve, often cause comparatively large and poorly reproducible heat effects, and are less useful when only minute quantities of heat are evolved. Batch reaction and solution calorimeters normally have a gas phase which may be the source of complications, especially in microcalorimetry. In flow calorimetric vessels, there is usually no gas phase, and they are therefore particularly suitable for mixing and dissolution experiments where significant changes in the composition of a gas phase can occur. Another advantage with flow and injection techniques is that they are often well suited to automatization.

ISOTHERMAL BIOCALORIMETRY

Aqueous solutions of simple model substances

Life cannot exist without water. Studies of the properties of water and the mutual interactions between water and biochemical substances and their simple models form a central part of biothermodynamics.

In thermodynamic work on simple model compounds, certain features of complex biochemical substances are simulated. Work on very simple compounds has the advantage that the influence of well-defined properties on the thermodynamic values can be studied in considerable detail. Results of such work have greatly influenced our views about, e.g., structural and reaction properties of biochemical systems. However, results from model studies should always be applied with great caution.

Current work on model systems is primarily directed to studies of weak non-covalent interactions, particularly water–solute interactions. The thermodynamic formalism in this field and results from studies of many groups of compounds, have been reviewed by Franks [5]. Model substances include the rare gases, hydrocarbons, various substituted hydrocarbons such as alcohols, carboxylic acids, esters, amines, amides, etc. Compounds forming the building blocks for biopolymers (amino acids, peptides, nucleotides and their constituents, sugars) are also important as well as lipids including micelles and liposomes. In order to explore the unique properties of water, it is frequently important to extend the investigations to non-aqueous solvents or to mixtures of organic solvents and water. Such solvents are also used as models for the environment in the interior of a biopolymer molecule or of a lipid phase.

The most basic calorimetric model experiments are those where pure compounds are transferred to infinitely dilute solutions. Such measurements are normally conducted with precise macrocalorimeters, using dissolution experiments, often combined with dilution measurements. Dilution experiments conducted over a suitable concentration range can also lead to values for solute–solute interactions, h_{xx}, h_{xxx} (see, e.g., refs. 5, 7 and 8).

Calorimetric experiments lead to values for ΔH and ΔC_p. However, if calorimetric enthalpy values are available, the entropy changes are usually best obtained by a combination of results from equilibrium measurements and calorimetric enthalpy values (cf. ref. 6).

Vaporisation enthalpy values are an important prerequisite for evaluation of the properties of the solvated state of molecules. Very few calorimetric vaporization studies have been performed so far, however.

From the difference between values for the dissolution into different solvents, values for the transfer of the solute between the solvents are obtained. These transfer processes are often looked upon as simple models for ligand biopolymer binding processes. It is sometimes essential to perform dilution experiments leading to values for the infinite dilute solution.

Heat capacity values obtained from solution calorimetric work ($\mathrm{d}\Delta H/\mathrm{d}t = \Delta C_p$) are of major importance in biochemical model compound studies. However, ΔC_p values reflect the properties of the initial as well as the final system for the dissolution process. If interest is focused on the solvated state, the partial molar heat capacity for the solute (at infinite dilution), $C_{p,2}^{\infty}$, is a more useful property. This can be determined by adding the corresponding

$\Delta C_{p,2}^{\infty}$ values to the value for the pure compound, C_p^*

$$C_{p,2}^{\infty} = \Delta C_{p,2}^{\infty} + C_p^* \quad (1)$$

Accurate C_p^* data are often lacking, even for very simple compounds at 25°C. Precise "drop" microcalorimeters [1,6] have proved suitable for such determinations, as well as for heat capacity determinations of dilute solutions leading directly to $C_{p,2}^{\infty}$ values. The very precise flow heat capacity calorimeter designed by Picker [1,6], available commercially through Setaram (France), is particularly suitable for this type of measurements.

During recent years, biochemical model work has to a large extent involved studies of hydrophobic hydration. The "hydrophobic effect" is believed to play a significant role in the structural stability and specificity of biological systems. From a calorimetric point of view, it is the heat capacity change which is of special interest. Hydrophobic hydration is characterized by a large increase in heat capacity, e.g., for a liquid hydrocarbon $C_{p,2}(aq)$ is about 3 times higher than the value for the pure liquid. The $C_{p,2}(aq)$ values change slowly with temperature and show remarkable additivity [9], particularly for nonionic compounds. Large negative ΔC_p values for biochemical processes are sometimes taken as a sign of the formation of "hydrophobic bonds", but caution should be exercised when making such statements. It is of interest in this connection that sugars and sugar alcohols also show very high $C_{p,2}(aq)$ values [10].

For the development of theoretical concepts about hydrophobic interaction, experimental data for hydrocarbons, rare gases and other slightly soluble compounds are of particular importance. Two microcalorimetric solution vessels designed for such studies are briefly described below.

Biochemical systems

Current calorimetric work on biochemical compounds is mainly concentrated on proteins, nucleic acids and membrane materials, and on assemblies of such compounds. Other substances of interest include intermediates of metabolic pathways and various key compounds, such as co-enzymes, vitamins, hormones, and energy-storage substances, but these groups of compounds have not yet been the subjects of systematic study. A few attempts have been made to characterize cell organelles such as ribosomes and mitochondria by calorimetric methods.

Studies of the binding of low molecular ligands to proteins and nucleic acids, and specific association reactions involving biopolymers, are of particular interest in isothermal biocalorimetry. The specificity is often very high, meaning that the binding processes are well defined. The ligands are frequently simple compounds and these processes therefore often appear to be comparatively simple and possible to analyse in some detail [11–13]. They are also well suited to microcalorimetric techniques and in many instances it

is possible to determine both equilibrium constants and enthalpy values, and thus entropy values as well.

There have been significant recent advances in microcalorimetric titration techniques. A titration attachment has been developed for the LKB batch microcalorimeter [14] (LKB Produkter, Bromma, Sweden). The technique can be automated easily and titration curves can now be produced much faster and with less material. In addition, the new technique gives significantly more precise results (cf. ref. 15).

Gill and co-workers [16,17] have recently described very sensitive microcalorimeters which are operated automatically and are particularly suitable for ligand binding work.

As part of a new four-channel microcalorimeter system [18], a flow-mixing calorimeter has been designed. This is significantly more sensitive than our earlier flow microcalorimeter [19]. The new system (described below) also incorporates a microtitration vessel [20].

Cellular systems

There is an increasing number of investigators who do microcalorimetric work on microorganisms, tissue cells and other cellular systems and on small animals. Such work is often directed towards practical applications in fields such as biotechnology and clinical analysis. Reviews on microcalorimetric work on different cellular systems are found in refs. 2 and 3. Sources of systematic errors and other problems connected with such studies were discussed in ref. 4.

The thermal power evolved in microcalorimetric measurements on small animals and growing microorganisms is frequently quite large, typically in the range of $\geqslant 100\ \mu W\ ml^{-1}$. However, in measurements on cultured tissue cells or cells isolated from humans or animals, the total thermal power is often only a few micro-Watts per millilitre of cell suspension, and very sensitive instruments must be used. It is often desirable to stir the cell suspensions, to add reagents during the measurements or to perfuse medium through vessels containing pieces of tissue or cells adhering to a support. The new "titration-and-perfusion vessel" [20] decribed below has been designed with such needs in mind.

SOME RECENT DEVELOPMENTS IN MICROCALORIMETRIC INSTRUMENTATION

Several microcalorimeters of the thermopile heat-conduction type have been designed in our laboratory, see, e.g., ref. 1. They were built for different types of measurements, but their basic mechanical design was closely similar in all cases. The experience gained with them and their commercial versions (LKB Produkter, Bromma, Sweden) has confirmed that a modular approach

in the design is most valuable, as it allows wide functional flexibility with a relatively small number of different mechanical and electrical units.

Some microcalorimetric techniques recently developed in our laboratory will now be briefly described.

New techniques for dissolution of slightly soluble compounds

Our interest in solution thermochemistry has for many years been directed towards studies of "hydrophobic hydration", i.e., the interaction between hydrophobic groups and water. Most of this work has been conducted on a macro level. However, for slightly soluble compounds—which are naturally of particular interest in this connection—it has been necessary to develop more sensitive microcalorimetric methods. A review of this group of instruments was recently reported [21]. Here the designs will only be very briefly described. Methods have been developed for slightly soluble liquids [22,23], gases [24], and solids [25]. In all cases, the instruments consist of rod-shaped insertion vessels which fit into our earlier ampoule calorimeter [26] or the new four-channel instrument system described below.

The vessel used for dissolution of liquids [23] is shown in Fig. 1. It consists of two concentric glass tubes with two heat-conducting regions in thermal contact with the surrounding water thermostat (d) and the calorimeter heat sink (e). Below these regions, there is a brass cup in thermal contact with the thermopiles. The cup contains a system of spiralized tubes (not shown in the figure) embedded in Woods' metal. These tubes form the dissolution region. During an experiment, a constant flow of solvent is sucked through the vessel at about 40 ml h^{-1} and thermostated during its passage down to the dissolution region. The solute is introduced into the dissolution zone by means of a thin steel tube fastened to a microsyringe. Typically, 5 μl of solute are added in each experiment. The measurements are performed automatically through the use of a microprocessor. The instrument can also be used for measurements of easily soluble liquids.

The vessel used for solution of gases [24] is quite similar, although the dissolution tube system is different. With this instrument, we have recently performed measurements at different temperatures on helium, neon, argon, krypton, xenon, methane, ethane, propane and 1-butane [27].

Our instrument for solution of slightly soluble solids [25] is made of stainless steel, but is in many respects similar to the vessels used for liquids and gases.

A four-channel microcalorimeter system

A "multi-channel" instrument has frequently been called for, e.g., in bio-analytical experiments, to speed up the rate of sample throughput in serial analysis. In work on living cellular materials, it is frequently desirable

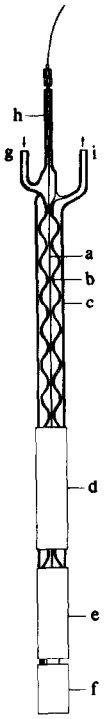

Fig. 1. Calorimetric vessel for dissolution of small quantities of easily or slightly soluble liquids: (a) steel tube attached to a Hamilton syringe; (b, c) concentric glass tubes; (d, e) brass tubes; (f) brass tube containing the dissolution vessel; (g) solvent inlet; (h) steel tube fixed to the central glass tube; (i) solvent outlet. (From ref. 23.)

to perform simultaneous comparative measurements from batches of cells in identical or in different reaction vessels. Such considerations led to the design of a new calorimetric system [18] briefly described below. It forms the prototype of the LKB "BioActivity Monitor" (LKB Produkter, Bromma, Sweden). To some extent, our new system is based on our earlier series of instruments [1]. A prime requirement has been to reduce the size of the calorimetric heat sinks so that one thermostatic bath of a manageable size can house more than one instrument. This, and the modular concept adopted, have led to a mechanical design quite different from the earlier instruments. The reduced size of the heat sinks has required a significant improvement in the stability of the thermostatic bath. An overall view of the instrument is shown in Fig. 2.

A thermostated water bath can hold up to four independently operated calorimeters ("channels"). The water thermostat is of the overflow type, where water is circulated by a centrifugal pump. Its temperature is regulated on two levels, in the centrifugal pump and in the bath outlet tube. Thermis-

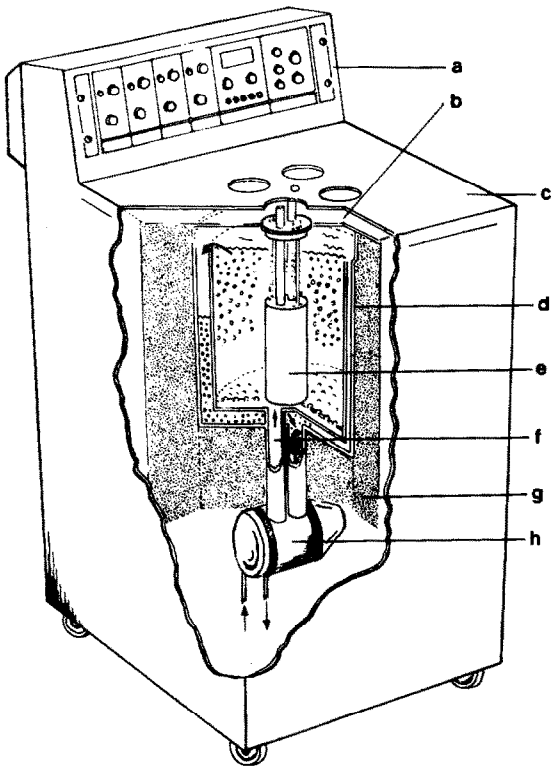

Fig. 2. The multichannel instrument: (a) electric console; (b) inner lid; (c) outer lid; (d) water bath; (e) twin calorimeter; (f) pump outlet tube; (g) polyurethane foam insulation; (h) centrifugal pump. (From ref. 18.)

tors are used as sensors. The temperature stability of the bath is $\pm 1 \times 10^{-4}$ K over long periods of time (days) if the room temperature does not fluctuate more than $\pm$ 1 K.

The calorimeters can be of different designs and can have different functions, but so far they have always been of the thermopile heat-conduction type. Figure 3 is a schematic illustration of the design of twin calorimetric channels used with cylindrical insertion vessels (diameter 14 mm).

A cylindrical stainless-steel vessel (f) contains an aluminium block assembly which serves as the calorimetric heat sink. This consists of two main cylindrical blocks (h and j) and four small, nearly cubic blocks (i) which are in contact with the thermopile. The twin calorimetric unit consists of two holders for insertion vessels ("ampoule holders") (l). Each ampoule holder is surrounded by two Peltier-effect plates (m) which form thermal bridges to the small aluminium blocks (i). For each ampoule holder, the Peltier-effect plates are electrically connected in series, whereas the two thermopiles thus formed are connected in opposition, giving a differential voltage signal from the twin calorimetric unit. The upper and lower parts of the ampoule holders

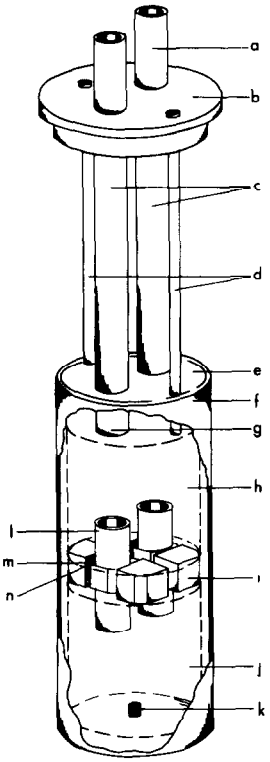

Fig. 3. Twin calorimetric channel used with cylindrical insertion vessels: (a) connecting tubes; (b) lid; (c) 16-mm steel tubes; (d) 6-mm steel tubes; (e) steel lid; (f) steel vessel; (g) connecting tubes; (h) aluminium block; (i) small aluminium block; (j) aluminium block; (k) steel spring; (l) ampoule holder; (m) Peltier-effect plate. (From ref. 18.)

fit into wide cylindrical bores in the main aluminium blocks. Above the ampoule holders the bores are narrowed in order to give a close fit with the calorimetric insertion vessels. The bores are carefully aligned with the two large steel tubes (c) of the lid assembly. These tubes and the narrow parts of the bores serve as heat exchange zones for the insertion vessels. Several types of simple insertion vessels are used: 1-, 3- or 5-ml cylindrical steel ampoules closed with screw lids and teflon packings and a commercially available glass vial. The baseline drift during 12 h and the maximum short-term deviation from the baseline are typically less than 0.1 μW.

In another type of channel, the twin calorimetric unit is fitted with two permanently installed flow vessels used for mixing and flow-through experiments, respectively. Both vessels consist of 24 carat gold tubes (ID 1 mm) wound on threaded aluminium tubes. Except for the thread, the aluminium tubes are closely similar to the ampoule holders shown in Fig. 3 and the tubes can thus also serve as holders for insertion vessels. The flow-mixing instrument has recently been used to obtain, for instance, precise measurements of h_{xx} and c_{xx} in H_2O and D_2O at low concentrations [28].

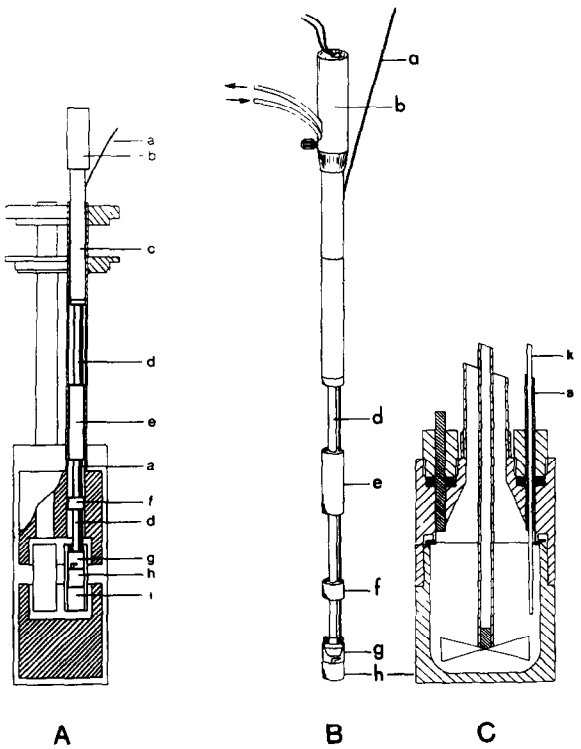

Fig. 4. Insertion vessel used for perfusion and titration experiments: (A) simplified picture of the vessel in measurement position; (B) the vessel; (C) section through the sample compartment (1-ml sample cup); (a) injection tube; (b) stirrer motor; (c) plastic tube; (d) steel tube; (e, f) brass bolts; (g) upper part of sample compartment; (h) sample cup; (i) aluminium cylinder; (k) injection needle. (From ref. 20.)

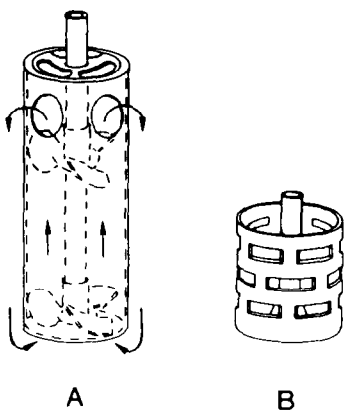

Fig. 5. Examples of stirrers attachable to the stirrer shaft: (A) turbine stirrer used with 3 ml vessel; (B) "cage" sample holder for pieces of tissue. (From ref. 20.)

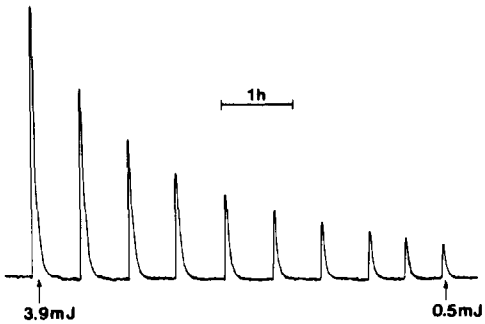

Fig. 6. Calorimetric record from the titration of ristocetin A with Ac-D-Ala. 7 μl of Ac-D-Ala solution (0.5 μmol) was injected in each step. (From ref. 20.)

In addition to the twin calorimetric channels, we have also built a single calorimetric channel used with 20-ml steel or glass ampoules (mainly designed for use with small animals). In this case, one large Peltier-effect plate is placed horizontally on the upper surface of the lower main aluminium block. An aluminium cylinder, which serves as the ampoule holder, rests on the Peltier-effect plate.

Very recently, a new insertion vessel was developed for the twin calorimeter shown in Fig. 3 [20] (Fig. 4). It is primarily intended for use with living cellular systems and in titration experiments. Experiments can be performed with or without medium perfusing through the vessel ($\leqslant$ 30 ml h^{-1}). It can be equipped with different sample cups made from stainless steel (volume

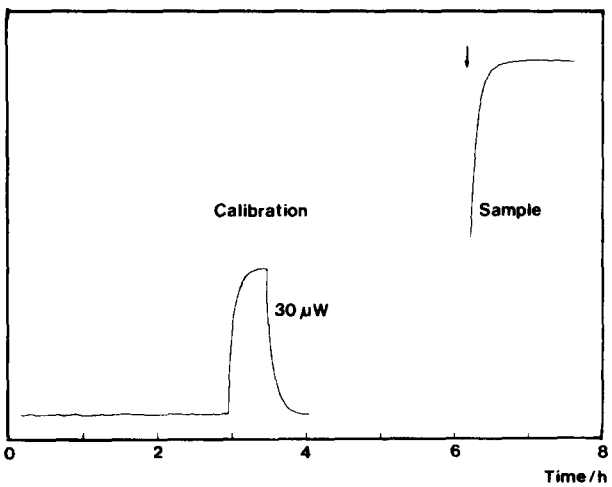

Fig. 7. Record from a baseline and calibration experiment followed by measurement of the thermal power from about 0.9×10^6 melanoma cells adhering to a polystyrene film. Medium perfusion rate was 10 ml h^{-1}. The arrow shows the time when the vessel was inserted into the measurement position. (From ref. 20.)

1–3 ml). Small quantities of reagents can be added to the sample compartment through injection needle(s) during the measurements. Different stirrers and sample holders have been tested. The turbine stirrer (Fig. 5) can be used with microcarriers and other relatively heavy suspensions. Stepwise calorimetric titrations can be performed by an automatic procedure. The vessel, like the calorimetric system, is conceived as a modular design. Its further development to incorporate new specialized functions is expected. The baseline stability is typically ± 0.3 μW over 10 h (stirring rate 50 rpm, perfusion rate $\leqslant 20$ ml h^{-1}).

Figure 6 shows the result of a titration experiment where 0.5 μmol of Ac-D-Ala is added stepwise to 0.7 ml of a solution containing 0.6 μmol ristocetin A (an antibiotic). The injections were made with a motor-driven syringe. Additions were regulated by use of a microprocessor, which was also used for the calculation of the voltage–time integrals. Values for the equilibrium constant and enthalpy change were calculated from the results.

Figure 7 shows results of an experiment with very active melanoma cells adhering to polystyrene film inserted into a 3-ml sample cup. A baseline was first recorded. A calibration experiment was then carried out at the same perfusion rate as in the subsequent experiment with the cells. The thermal power evolved was 72 μW, corresponding to about 80 pW per cell.

REFERENCES

1 C. Spink and I. Wadsö, Methods Biochem. Anal., 23 (1976) 1.
2 M.N. Jones (Ed.), Biochemical Thermodynamics, Elsevier, Amsterdam, 1979.
3 A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, London, 1980.
4 I. Wadsö, in A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, London, 1980, pp. 247–273.
5 F. Franks, in M.N. Jones (Ed.), Biochemical Thermodynamics, Elsevier, Amsterdam, 1979, pp. 15–74.
6 I. Wadsö, Pure Appl. Chem., 52 (1980) 465.
7 H.L. Friedman and C.V. Krishnan, J. Solution Chem., 2 (1973) 119.
8 F. Franks, M. Pedley and D.S. Reid, J. Chem. Soc., Faraday Trans., 1 (1976) 359.
9 N. Nichols, R. Sköld, C. Spink, J. Suurkuusk and I. Wadsö, J. Chem. Thermodyn., 8 (1976) 1081.
10 Y.-N. Lian, A.-T. Chen, J. Suurkuusk and I. Wadsö, Acta Chem. Scand., Ser. A, 36 (1982) 735.
11 M. Eftink and R. Biltonen, in A.E. Beezer (Ed.), Biological Microcalorimetry, Academic Press, London, 1980, pp. 343–412.
12 H.-J. Hinz, Annu. Rev. Biophys., 12 (1983) 285.
13 P.D. Ross and S. Subramanian, Biochemistry, 20 (1980) 3096.
14 A.E. Beezer, W.H. Hunter, R.P. Lipscombe, R.D. Newell and D.E. Storey, Thermochim. Acta, 55 (1982) 345.
15 A.-T. Chen and I. Wadsö, J. Biochem. Biophys. Methods, 6 (1982) 307.
16 R.B. Spokane and S.J. Gill, Rev. Sci. Instrum., 52 (1981) 1728.
17 J.R. McKinnon, L. Fall, A. Parody-Morales and S.J. Gill, Anal. Biochem., 139 (1984) 134.
18 J. Suurkuusk and I. Wadsö, Chem. Scr., 20 (1982) 155.

19 P. Monk and I. Wadsö, Acta Chem. Scand., 22 (1968) 1842.
20 M. Görman Nordmark, J. Laynez, A. Schön, J. Suurkuusk and I. Wadsö, J. Biochem. Biophys. Methods, 10 (1984) 187.
21 I. Wadsö, Thermochim. Acta, 88 (1985) 169.
22 S.J. Gill, N. Nichols and I. Wadsö, J. Chem. Thermodyn., 7 (1975) 175.
23 S.-O. Nilsson and I. Wadsö, J. Chem. Thermodyn., 16 (1984) 317.
24 S.J. Gill and I. Wadsö, J. Chem. Thermodyn., 14 (1982) 905.
25 S.-O. Nilsson and I. Wadsö, to be published.
26 I. Wadsö, Sci. Tools, 21 (1974) 18.
27 G. Olofsson, A.A. Oshodi, E. Qvarnström and I. Wadsö, J. Chem. Thermodyn., 16 (1984) 1041.
28 D. Hallén, S.-O. Nilsson and I. Wadsö, J. Chem. Thermodyn., in press.