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A system of microcalorimeters

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Abstract

For more than ten years our laboratory has been engaged in extending a modular microcalorimetric system designed for accurate work in several areas of basic and applied chemistry and biology. The system includes a very stable thermostatted water bath that can house up to four twin heat-conduction microcalorimeters. The calorimeters are used with various flow vessels or insertion vessels ranging from simple closed ampoules to stirred vessels fitted with injection tubes, devices facilitating dissolution processes (l, g, s), electrodes (pH, $p(O_2)$), optical cables or equipment for electrochemical processes.

INTRODUCTION

The optimal design of a calorimeter is governed by many factors. Even for instruments specialized for a certain type of measurement, for example reaction calorimeters used with aqueous systems at ambient temperatures, several experimental parameters may have a major influence on the design: sensitivity and accuracy required, aggregation state(s) of the reagent(s), preferred method of initiation of the experiment, reaction rate, stirring of reaction mixture, etc. If biological systems are involved, there are often requirements for special physiological conditions and these might also influence the instrument design. This large variation in the need of special instrument properties does create problems, not the least economical. However, if a modular concept is used, it is usually possible to incorporate several specialized measurement functions in a system of calorimeters where most parts of the different instruments are identical. Three such modular systems of calorimeters have been developed at our laboratory: a series of quasi-adiabatic (isoperibolic) macrocalorimeters (ref 1), a group of heat conduction microcalorimeters (ref 1) and the system of microcalorimeters that will be presented here. Following a first report (ref 2) where the basic units of the present system were described, our laboratory has been engaged in the extension of this system during more than ten years. As for our earlier microcalorimeters (ref 1), the heat conduction principle is employed and semiconducting thermopiles, 'Peltier effect plates', are used as sensors for the heat flow between calorimetric vessels and surrounding heat sinks. Twin calorimeters are used in most cases.

The basic units

The basic units consist of a precisely thermostatic water bath with up to four twin calorimeters and an electronic console, Fig. 1 A, (ref 2). The twin calorimeters are used together with insertion vessels of different designs and functions. The water bath is of the overflow type where the water is circulated by a centrifugal pump placed under the bath and the temperature is regulated at two levels. The pump temperature is controlled by a thermistor positioned in the pump house and cooling or heating power is delivered by water-cooled Peltier effect plates. The thermistor used as the main temperature controller is placed in the upper part of the bath inlet tube and an insulated heater spiral is positioned in the bath outlet tube in direct contact with the water. The liquid volume of the bath is 20 l and the water flow rate is 30 l·min^{-1} . The bath and the two tubes from the pump are insulated by a 10 cm thick layer of foamed polyurethane. When used in the ambient temperature range, the stability of the bath is $\pm$ 1·10^{-4} K over long periods of time (days) provided that the room temperature does not fluctuate more than $\pm$ 1 °C. The bath can be used at up to 90 °C with water as liquid medium, and can hold up to four twin calorimeters

('channels') of the types shown schematically in Fig. 1. Those channels are used with cylindrical insertion vessels with an outer diameter ∅ = 14 mm. Some of our more recent vessel designs have ∅ = 28 mm and require larger channel diameters. Typically, one large channel is then used with two smaller channels in the same bath. The properties of different channels equipped with different number and type of thermocouple plates were recently discussed (ref 3).

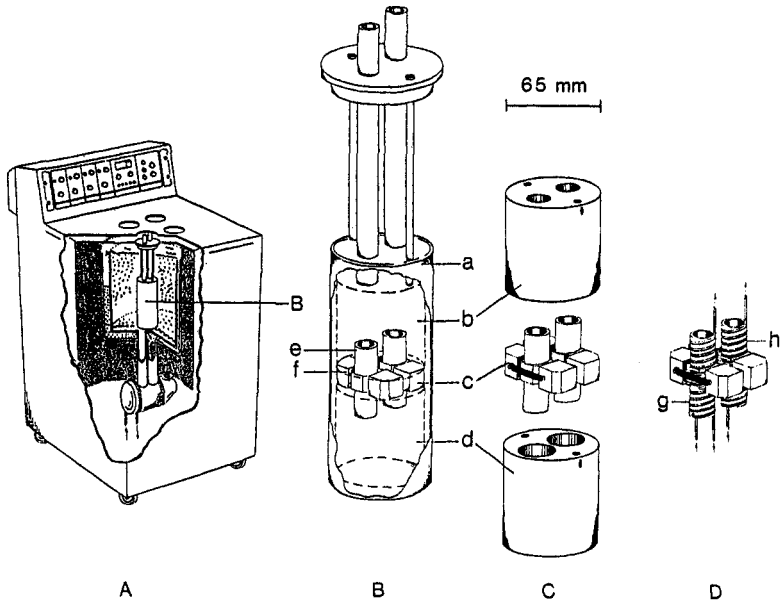

Fig. 1. Basic units of the calorimetric system. A: The thermostatted water bath and electronic console. B, C: Twin microcalorimeter; a, steinless steel can; b, c, d, aluminium blocks; e, ampoule holder; f, thermopile (Peltier effect plate). D: Flow calorimetric unit (which may be used with insertion vessels). g, mixing vessel; h, flow-through vessel.

CALORIMETER VESSELS

Static ampoules

In particular for stability measurements on products of technical importance and in some experiments with biological samples we use simple sealed cylinders made from acid-proof steel, volume 1 - 4 cm^3 (∅ = 14 mm) or 15 - 28 cm^3 (∅ = 28 mm). Alternatively, disposable glass vessels are used. The steel vessels are sometimes coated with teflon on the inside in order to improve the resistance against corrosion. The vessels are inserted into the 'ampoule holders' (Fig. 1 e; aluminium tubes in thermal contact with the thermopiles) by use of thin steel tubes fitted with horizontal disks serving as reflectors. The stepwise introduction allows the ampoule to equilibrate in two or three positions which requires about 45 min if the measurements are made at high sensitivity. When identical ampoules are placed in both vessel holders of the small channel, the baseline stability is typically better than ± 0.1 μW during 12 h (ref 2). For the larger channel the corresponding value is usually about ± 0.2 μW. These static vessels are mainly used in measurements where stirring and injection are not required.

Flow vessels

Fig. 1 D shows a twin calorimetric unit where spiralized gold tubes surround the ampoule holders which can therefore act as combined flow vessels and holders for insertion vessels (ref 2). One of the flow vessels, a mixing vessel, has two inlet tubes, a mixing point, and an outlet tube. The other vessel is a flow-through vessel. The heat exchange coils through which the liquids are introduced are not shown in the figure. Normally, when one of the flow vessels is used the other serves as an inactive reference. The vessel arrangement shown in Fig. 1 D thus has three different functions. When used with insertion ampoules, the baselines usually are slightly less stable than those obtained with the simpler calorimetric unit shown in Fig. 1 C. Other types of flow vessels, 'perfusion vessels' which can be fitted with stirrers, are discussed below.

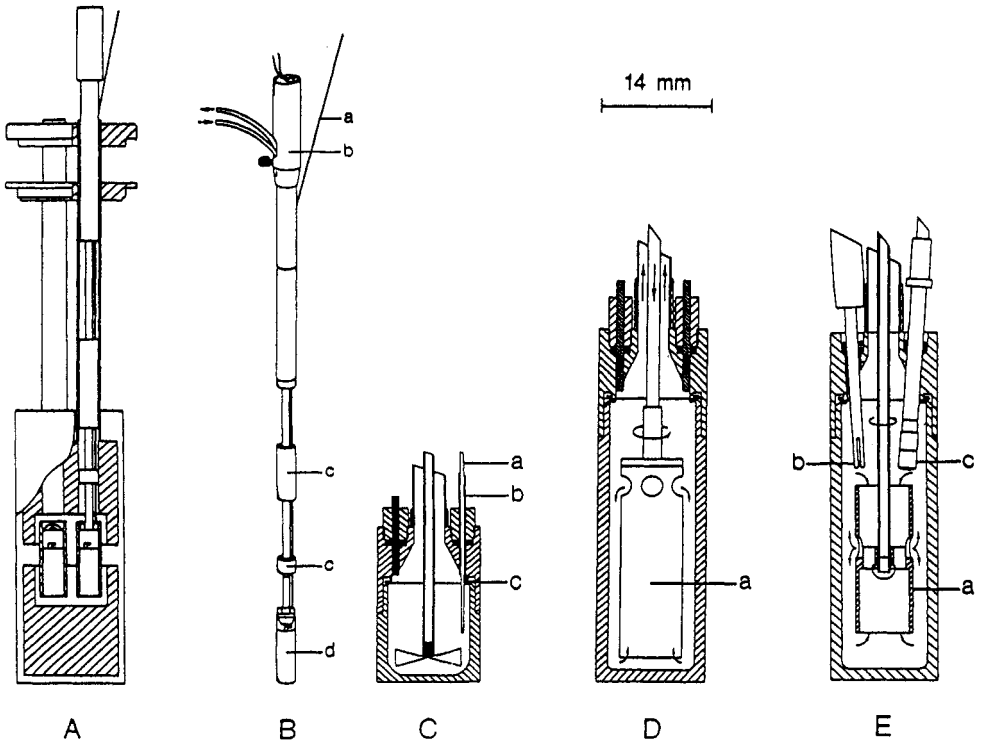

Fig. 2. Insertion vessels. A: Simplified picture of titration-perfusion vessel in measurement position. B: Picture of the vessel indicated in Fig. 1A. a, guide tube; b, stirrer motor; c, brass bolt; d, sample compartment. C: Sample compartment of 1 ml titration vessel. a, injection needle; b, guide tube; c, teflon seal. D: Sample compartment of 3 ml perfusion vessel. a, turbine stirrer. E: Sample compartment of 3 ml vessel fitted with electrodes. a, turbine stirrer; b, pH-electrode; c, oxygen electrode.

Titration–perfusion vessels

Several slightly different titration - perfusion vessels (insertion vessels) have been designed for the small (ref 4) - as well as for the large - channel type. One vessel (∅ = 14 mm) and three different sample containers (volume 1 and 3 ml) are shown in Fig. 2. The sample containers are identical with those used with the simple static ampoules. Our titration - perfusion vessels were mainly designed for two purposes: as titration vessels in studies of ligand-binding processes and as reaction vessels for suspensions of living cells. The liquid content can be stirred by different kinds of stirrers (Figs 2, 3) which also might serve as sample holders. Experiments can be made with a gas phase present or the sample compartment may be completely filled with solution or suspension.

Precise additions of reagents, typically a few mm^3, can be made through a very thin needle (i. d. 0.15 mm) introduced through a steel guide tube (a in Fig. 2 B). Compression effects are avoided by means of a small air channel between the injection needle and the guide tube.

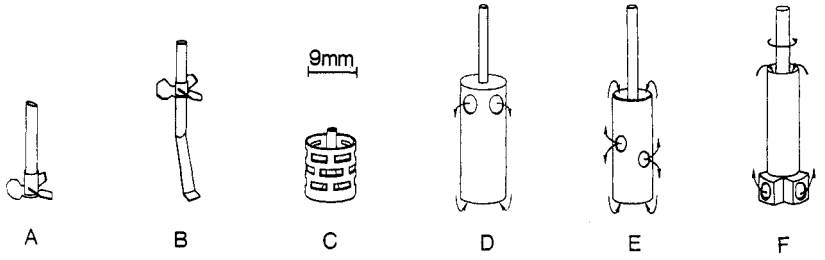

Fig. 3. Different types of stirrers used with insertion vessels. A, simple propeller; B, 'spoon-stirrer' with propeller; C, cage made from Kel F for use with tissue pieces; D, E, and F, different models of turbine stirrers giving different flow patterns.

Heat conduction calorimeters have many excellent properties such as high sensitivity, very good long-term stability, and a comparatively simple mechanical and electronic design. Their main disadvantage is the slow thermal response, *i.e.* the large time constants. When the 1 ml vessel shown in Fig. 2 C is used in a titration experiment involving about 15 injections interspaced with baseline observation periods, the measurement will require about 10 h. However, we have recently shown (refs 3, 5) that such experiments can be conducted about ten times faster, without loss of accuracy, by use of a 'dynamic correction' technique. With this method, consequtive injections are made at short intervals, ca 5 min, and the differential potential signal from the thermopile will thus not return to the baseline level between the injections. After the experiment, the calorimetric wave-shaped potential - time curve can be deconvoluted to a baseline interrupted by sharp injection peaks, Fig. 4.

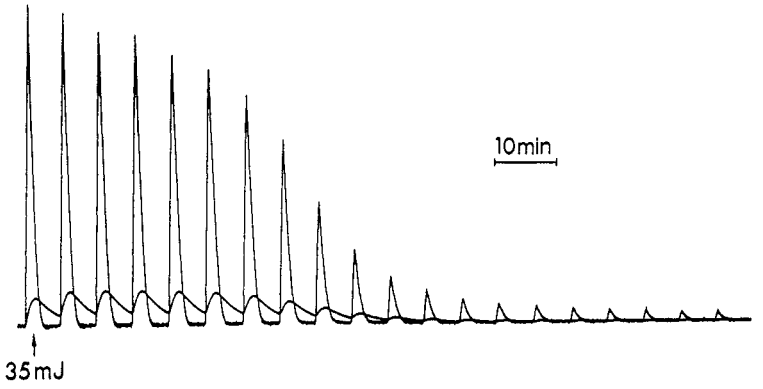

Fig. 4. Calorimetric record from a stepwise titration experiment using the Ba^{2+} - 18-crown-6 test reaction (ref 20). The horizontal baseline, interrupted by sharp injection peaks, was obtained by 'dynamic correction' of the wave-shaped experimental potential-time curve.

Sometimes biological samples, for example pieces of tissues, are best measured under conditions where medium is constantly perfused through the vessel. The hollow stirrer shaft and the annular space outside it (see Fig. 2 D) then act as a countercurrent heat exchanger allowing a maximum flow rate of about 25 $cm^3 \cdot h^{-1}$.

Vessels of the type shown in Fig. 2 can also be used for mixing of liquids that are not easily mixed in flow-mixing vessels made from tubes (Fig. 1 D). The sample cup should then be charged with the reaction mixture and the two components are added using two insertion needles. Alternatively, the two reagents can be added through the shaft and the annular space outside the shaft, respectively.

In work with cellular systems and in some titration experiments, it can be of great advantage to equip a titration - perfusion vessel with electrodes (ref. 6). Fig. 2 E shows a 3 ml vessel with two miniaturized electrodes: a pH electrode and a Clark cell for determination of O_2-concentration. The electrode measurements do not interfere with the calorimetric signals.

Vessels of the general type shown in Fig. 2 have been used also in experiments involving adsorption of solutes on solid materials. In particular a version with a 20 cm^3 sample container ($\varnothing$ = 28 mm) has proved to be suitable in such experiments. The vessels can then be used either in the titration mode or as perfusion vessels. In the latter case, the effluent solution can be used for continuous analytical determination of solute concentration leading to values for adsorption isotherms. Rather large particles (> 0.3 mm) can often be well exposed to the solution if they are kept in some sort of rotating cages (*cf* Fig. 3 C). However, it may be necessary to work with fine powders in order to obtain large enough signals. It is then often possible to keep the powder in good contact with the solution by use of a suitable stirrer, *e.g.* a 'spoon' stirrer (Fig. 3 B) or some type of 'turbine' stirrer (Figs 3 D - F). The choice of stirrer is often the most critical factor in such experiments.

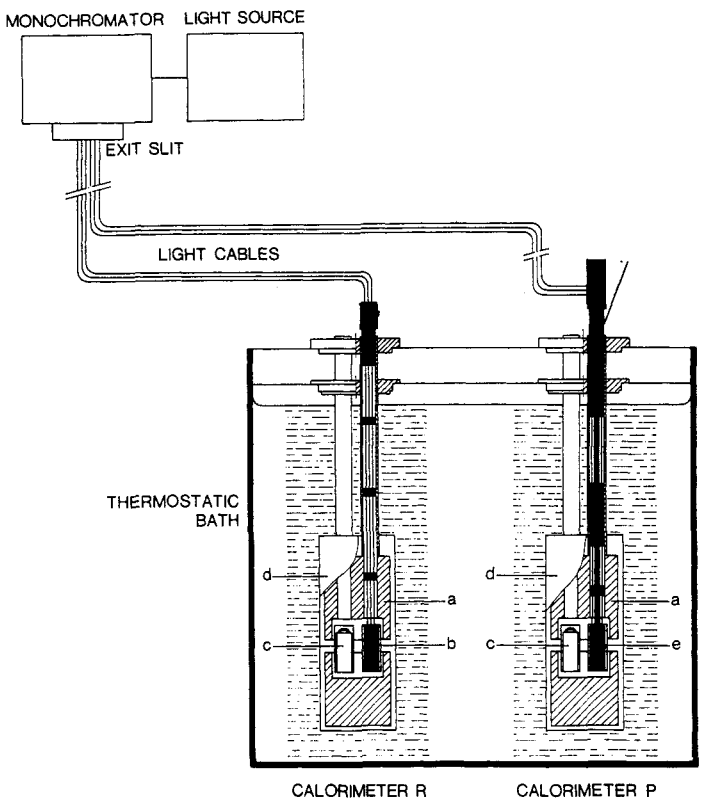

Fig. 5. Schematic picture of the photocalorimetric system. Two twin heat-conduction calorimeters (calorimeters P and R) were immersed in a thermostatic bath. Calorimeter P was used for the photochemical experiments and calorimeter R served as a photoinert reference. Light from a monochromator was supplied to each of the photocalorimetric vessels by use of three light cables. For each calorimeter: c, a reference 'vessel'; d, steel can; and a, aluminium heat sink. b, photoinert light absorption vessel; e, photochemical reaction vessel. The thermopiles and parts of the heat sink surrounding the vessels b, c, and e are not shown. The differential signal from each calorimeter was recorded.

Light-induced processes are important both in chemistry and in biology and this has led us into the field of photochemistry (ref 7). Figure 5 shows schematically our photocalorimetric system which employs two twin microcalorimeters using 14-mm vessels. The reaction vessel of calorimeter P, similar to that shown in Fig. 2, is used for photochemical or photobiological experiments and calorimeter R serves as a photoinert reference. Light from a monochromator is supplied to each of the calorimetric vessels by use of optical cables. During a photocalorimetric experiment, the photoinert calorimeter R will provide a continuous record of the luminous power introduced into the reaction vessel of calorimeter P. We are presently testing a new photocalorimetric system similar to that shown in Fig. 5 but where the vessels in calorimeter P have ∅ = 28 mm (ref 8). The photochemical vessel can then be equipped not only with optical cables and an injection device but also with electrodes like those in the vessel in Fig. 2 E.

For many years a significant part of the research at our laboratory has been concerned with investigations of solute - solvent interactions, in particular hydrophobic hydration, *i.e.* interactions between water and hydrophobic compounds or groups. For this reason we have been interested in measurements of enthalpies and heat capacities of dissolution in water as well as in other solvents. In some cases macro-solution calorimeters of the quasi-adiabatic type (ref 1) may still be the best instruments for such work. However, interesting compounds often have very low solubility which has led us as to develop a series of microcalorimetric dissolution vessels for gaseous, liquid and solid solutes. This work was initiated many

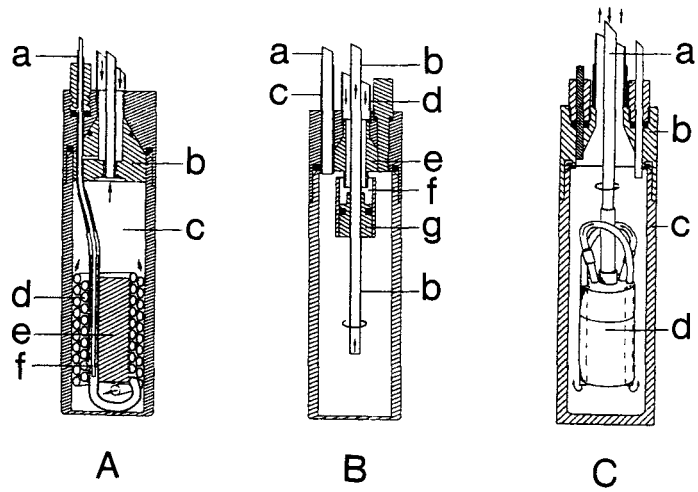

Fig. 6. Vessels for dissolution of liquids, gases, and solids. A: Section through the vessel for dissolution of liquids: a, guide tube; b, cone; c, hold-up volume; d, double-coiled gold or silver tube; e, silver core; f, hypodermic needle: B: Section through the vessel for dissolution of gases. a, hypodermic needle; b, stirrer shaft; c, guide tube; d, stainless steel tube; e, cone; f, water seal; g, stirrer. C: Section through the vessel for dissolution of solids: a, stirrer shaft; b and c, sample compartments; d, dissolution ampoule attached to the stirrer shaft a.

years ago (ref 9) and the vessel designs have gradually advanced to become components of our present system of microcalorimeters. The three vessels shown in Fig. 6 are simplified diagrams of titration - perfusion vessels (*cf* Fig. 2) modified to serve as dissolution vessels for small samples of liquid, gaseous and solid compounds, respectively (ref 10). The vessel for liquid solutes, Fig. 6 A (ref 11), can be used both with easily and slightly soluble compounds, whereas the vessel for gases (ref 12) and for solids (ref 13), Fig. 6 B and C, respectively, require slightly soluble samples. In all cases the solvent is made gas-free by gentle boiling in a still. In experiments with liquid solutes, an accurately known volume, about 3 mm^3, is injected into a coiled tube positioned in the sample container of the vessel. Slightly soluble solutes are retained in the coil but are smeared out by constant flow of solvent passing through the coil. The large contact area thus formed between solute and solvent will increase the rate of the dissolution process. The most suitable coil material varies for different kinds of solutes. So far, teflon, gold, siliconized glass or gold or silver alloys have been used. For easily soluble liquids, injections can be made stepwise or, preferably, continuously in steady state experiments where the final solute concentration will be well defined. For some solutes there will be a small but significant diffusion through the tip of the hypodermic needle during the time when the instrument baseline is established, but this effect can be corrected for (ref 14). The syringe used for the injections can be fitted with a mercury seal to prevent leakage of very volatile solutes (ref 14). For easily soluble liquids that are difficult to mix with the solvent it may be more suitable to use the original stirred titration - perfusion vessel (ref 4). In most cases with liquid solutes it is possible to reach a final solute concentration that can be considered as infinitely dilute.

In the gas dissolution experiments about 0.3 cm^3 of gas, measured accurately volumetrically, is injected into the sample container of the vessel (Fig. 6 B) where it is dissolved by a constant flow of gas-free solvent. As for the other dissolution vessels the solvent is sucked through the vessel by use of a peristaltic pump. The dissolution of the gas bubble is facilitated by rotation of the shaft - liquid seal device (l, n in Fig. 6 B). The liquid flow rate is about 25 cm^3/h. For very slightly soluble gases such as He and SF_6 the dissolution time is about 8 h for 0.2 cm^3 of gas. We have shown that this gas dissolution vessel can be used for simultaneous determination of solubility and enthalpy changes (ref 15).

In the vessel used for dissolution of slightly soluble solids (Fig. 6 C), a few mg of the sample is pressed to the bottom of a small cavity in a steel ampoule, d, until it forms a thin attached layer, e. The cavity forms part of a channel system connected to the hollow shaft. The cavity and the channels in the dissolution ampoule are filled with saturated solution at the start of an experiment. The rest of the perfusion system is filled with solvent. The dissolution process starts when solvent is sucked through the vessel. The

dissolution vessel is then rotated to equalize the temperature and the solute concentration in the vessel. It should be noted that this calorimeter measures the dissolution of a solid substance in equilibrium with its saturated solution. Very recently we have developed another microcalorimetric method by which dry, slightly or easily soluble solid samples can be dissolved (ref 16). With this new technique, up to four samples (1 - 3 mg) can be 'injected' consecutively into the large titration vessel (∅ 28 mm) fitted with a turbine stirrer.

Other vessels

A vaporization vessel (ref 17) and a gas adsorption vessel (ref 18) are presently being tested. Finally, we should mention a 25 ml vessel for measurements on electrochemical processes allowing gas evolution (ref 19). The vessel is part of the modular system and is partly identical to a large (∅ 28) titration vessel. The thermal power evolved is normally very high, typically in the order of 1 W (which is a very high value also for a 'macrocalorimeter'). The vessel was used with a single-vessel channel where the heat sink was in good thermal contact with the surrounding thermostatted bath.

CALIBRATIONS AND TESTS

Calorimeters are normally calibrated electrically. This is a convenient and also an accurate method from the point of view that values for electric power or energy can be measured accurately. However, many calorimetric vessels with specialized functions are often highly non-ideal from a calorimetric point of view. Further,sometimes it is not possible to use heaters that have an ideal design and are positioned in the most representative part of the vessel. It is, therefore, important to have available reliable and easy-to-use chemical processes suitable as test or calibration processes. We have recently discussed several such processes suitable for use with the present system of microcalorimeters (ref 20, 21).

CONCLUSIONS

The modular concept used for the present group of microcalorimeters has proved to be useful for applications in broad areas of basic and applied chemistry and biology. We expect that our laboratory will be engaged in the further extension of the system for some more years.

The basic units of this group of calorimeters (ref 2) together with several of the more specialized vessels, have been developed into commercial instrument systems by LKB Produkter and later by ThermoMetric AB (Järfälla, Sweden).

Acknowledgements

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