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A SCANNING MICROCALORIMETER FOR THERMALLY INDUCED TRANSITIONS IN SOLUTION

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ABSTRACT

A scanning microcalorimeter for the measurement of energies of transition in solution is described. The calorimeter utilizes semi-conductor thermoelectric modules and is of a very simple and inexpensive construction. The imprecision of measurement is three percent when measuring 25 to 250 mJ of heat associated with transitions over temperature intervals of up to 8 K. The calorimeter operates from ambient to 90°C.

INTRODUCTION

The accurate measurement of heat effects accompanying thermally induced transitions of substances in solution is a difficult problem when one deals with broad transitions occurring in dilute solution. Acquisition of such thermodynamic data is of scientific interest in that it contributes to our understanding of the molecular forces involved in such processes. The development of suitable instruments is a necessary prerequisite to such work, and several different types of calorimeters have been developed for this purpose[1].

The apparatus described herein, unlike the scanning calorimeters described in ref. 1, utilizes semi-conductor thermopiles which serve to reduce substantially the difficulty and cost of construction. It was also designed in such a way that it could be operated in any of several different modes, depending on the availability of the auxiliary electronic equipment. It is simple and inexpensive to build, highly sensitive, and easy to operate.

DESCRIPTION OF CALORIMETER

The major features of the calorimeter are shown in schematic cross-section in Fig. 1. The central block of the instrument is a copper right circular cylinder (10.1 cm

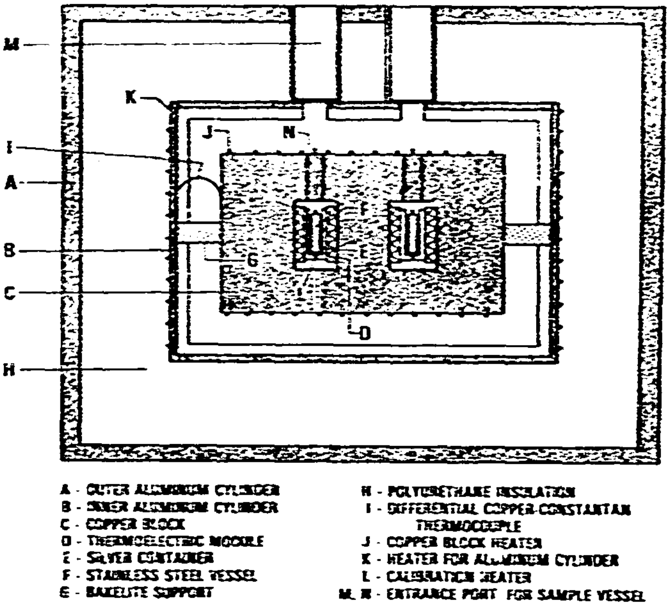

Fig. 1. Schematic cross-section of scanning microcalorimeter.

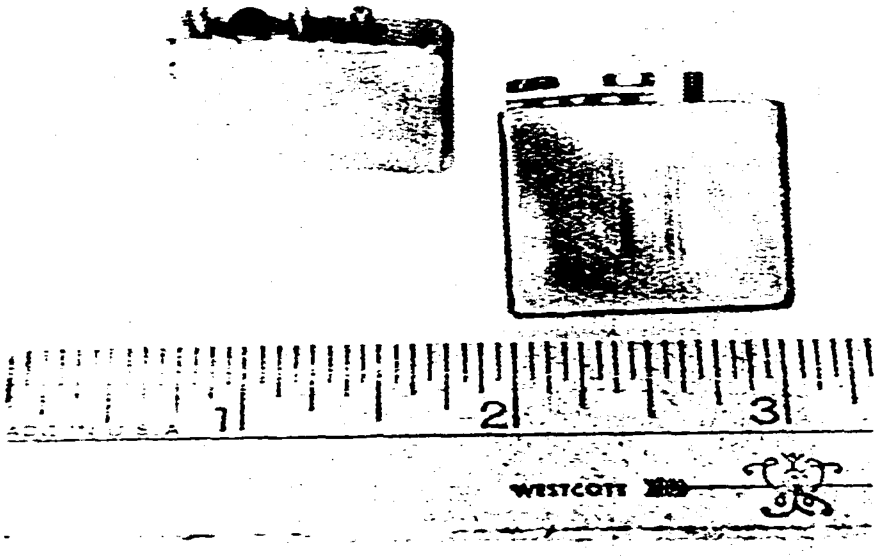

Fig. 2. Stainless-steel calorimeter cells.

diameter and 15.2 cm long) which is laterally sectioned into equal thirds. Each end section is fastened to the center section by four cap screws. In order to allow for possible differential operation, there are two rectangular cavities milled into the block equidistant from the end and perpendicular to its major axis. Each cavity accommodates a thermopile assembly, which consists of a pair of bismuth sellenide–bismuth telluride–bismuth antimonide thermoelectric modules (Model No. 3958-1, Cambion Corporation*)[2] electrically connected in series so that the resultant thermopile voltage is the sum of the voltages of the individual thermoelectric modules. Between the thermoelectric modules is sandwiched a silver rectangular container (3 cm wide by 3 cm high by 1.1 cm thick) which accommodates a rectangular stainless steel sample cell (2.7 cm wide by 2.2 cm high by 0.47 cm thick) with an internal volume of 1.8 cm^3. The sides of the cell are 0.079 cm thick. The calorimeter sample cells are shown in Fig. 2. A vapor-tight seal is made by tightening two No. 2-56 (national coarse thread) nuts bringing pressure to bear on a stainless-steel plate which compresses a No. 5-187 Buna-n O-ring seated around the top of the cell filling hole. The cell is provided with an additional No. 2-56 male stud for connection to a threaded rod which is used for installation of the cell within the calorimeter and for its removal for cleaning and loading.

The copper block is suspended by means of 1.2 cm diameter bakelite rods extending from its ends through the walls of a surrounding aluminum cylinder (21 cm diameter by 15 cm high, with wall thickness of 0.318 cm and 0.3 cm thick top and bottom plates); note that the axis of the copper block is perpendicular to the axis of the inner aluminum cylinder. A 0.6 cm thick layer of polyurethane foam covered with a layer of aluminum foil is cemented to the inner surface of the aluminum cylinder and faces the copper block. The inner aluminum cylinder is surrounded on all outer surfaces by foamed polyurethane, 5 cm thick, which is contained within an outer aluminum cylinder (32.4 cm diameter by 28.6 cm long, with wall thickness of 0.375 cm and 1 cm thick end plates). Holes are provided in the top surfaces of the inner and outer aluminum cylinders to permit the sample cells to be introduced into and removed from the copper block. The access channels in the copper block are sealed by copper plugs, while those through the aluminum cylinders contain polyurethane.

For raising the temperature of the block, a 100-Ω heater of No. 34 AWG glass insulated manganin wire is bifilarly wound around the copper block, two and one-half turns on each section. A 100-Ω heater is bifilarly wound on the outer lateral surface of the inner aluminum cylinder. Heaters for electrical calibration of approximately 100 Ω were made from No. 40 AWG formvar insulated manganin wire bifilarly wrapped on a copper spindle which was inserted in the base of each of the silver cell holders. These heaters were provided with current and potential leads of

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*Certain commercial materials and products are identified in this paper to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement either by the National Bureau of Standards or the National Institutes of Health.

No. 30 AWG copper wire, 4 cm of which was attached to the side of the silver cell as a thermal tie-down. The thermopiles, wires, and silver containers were glued down with adhesive (Scotch-Grip 1300, 3M Co.). A copper–constantan thermocouple is attached near the center of one side of the copper block for the purpose of determining its temperature. A differential copper–constantan thermocouple was placed between the copper block and the inner aluminum cylinder to be used as a sensor element for the automatically controlled environmental heater system. All of the heater, thermocouple, and thermopile leads were tied down on the same path for one turn about the copper block and then along the inner surface of the inner aluminum cylinder before being led out via small holes in the sides of the inner and outer aluminum cylinders to terminal strips mounted on the exterior of the instrument.

ELECTRONIC MEASUREMENT AND CONTROL EQUIPMENT

In order to determine the heat flux as a function of time from the copper block to the sample vessel, it is necessary to measure the thermopile voltage as a function of time. The thermopiles surrounding the two separate calorimeter cells are connected in electrical opposition (differential mode of operation) and the resultant voltage fed into a d.c. amplifier (Keithley Model 150B) whose output is recorded by a potentiometric chart recorder. A well-regulated, constant voltage d.c. power supply (Sorensen Model QHS40-.5) is connected to the copper block heater and its power is used to raise the temperature of the block. This temperature is determined by measurement of the voltage of a copper–constantan thermocouple referenced against an ice–water bath.

A substantial decrease in the scanning rate with increased temperature would occur during the course of an experiment if the temperature difference between the copper block and the inner aluminum cylinder were not minimized. The circuitry used to accomplish this purpose is as follows: The output of a differential copper–constantan thermocouple junctioned between the copper block and the inner aluminum cylinder, and which monitors the temperature difference between these two bodies, is fed to a null detector (Keithley Model 147), the output of which drives a current-adjusting type control unit having proportional, rate, and reset actions. The control unit output determines, via a transistor amplifier arrangement, the quantity of electrical power, from an unregulated d.c. power supply, delivered to the cylinder heater. The temperature difference between the copper block and the aluminum cylinder was found to be controlled to within one millidegree.

Electrical calibration of the calorimeter is performed by measuring with a digital voltmeter (Fairchild 7100A), the voltage drop across the calibration heater located in the silver cell holder and that across a 100-Ω standard resistor connected in series with it. Current is supplied from a constant-current d.c. power supply. The standard resistor and reference standard cell, used to check the accuracy of the digital voltmeter, were compared with other standards that had been calibrated by the National Bureau of Standards. The instrumental calibration constant, expressed in units of W V^{-1}, is measured as the ratio of the steady-state electrical power delivered via the calibrating heater to the steady-state voltage developed by the thermopile during calibration. The cell is loaded with the working volume of water (1.6 cm^3) during calibration. Since the calibration constant is a characteristic of the thermoelectric modules and their thermal contact with the silver box and copper block, it is not necessary to calibrate during every run, but only occasionally to insure that this instrumental parameter has not changed.

MODES AND PRINCIPLE OF OPERATION

By virtue of the dual cell construction, many modes of operation of this instrument are possible. For example, one might choose to perform a single ended run, in which the voltage of a single thermopile assembly is monitored, or to connect the thermopiles in series opposition to perform differential experiments. The possibility of employing electrical compensation of endothermic heat effects also exists for both single or differential modes of operation. The manner in which one chooses to run an instrument of this type may be based upon factors of convenience such as which configuration may yield the most linear and reproducible baseline, or on the availability of electronic apparatus.

The operation of scanning calorimeters of the heat-conduction type has been treated theoretically as a one-body model[3]. Insofar as that model contained certain simplifying assumptions, the possibility does exist that more sophisticated treatments may yet reveal unsuspected subtleties to be associated with the analysis of the raw data. Nevertheless, in the absence of heat capacity changes from the initial to the final state of the solution being investigated, the change in internal energy (ΔU) to be associated with a given transition is taken to be equal to the electrically measured calibration constant (units of W V^{-1}) multiplied by the peak area (units of V sec) associated with a given thermally induced transition. If there is a heat capacity change, additional correction terms become necessary[3].

CHARACTERISTICS, TESTS, AND PERFORMANCE OF CALORIMETER

The voltage signal produced by the input of a constant electrical power via the calibration heaters was measured at each thermoelectric module, with results indicating that, for the two thermopile assemblies, each of the two thermoelectric modules behaved nearly identically (0.3 and 2.5 percent difference in module response at the two respective thermopile assemblies).

The instrument calibration constant, determined electrically, is shown in Fig. 3 as a function of temperature. Imprecision of repeated calibrations was found to be 0.2% (estimated standard deviation of a measurement). We infer from experiments on heat-conduction calorimeters of similar design[4], but operated under isothermal conditions, that there should be only small error (less than 0.2%) from incorrect heater placement.

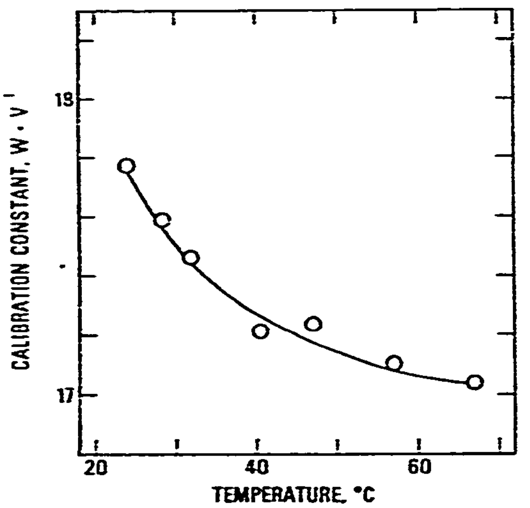

Fig. 3. Calibration constant as a function of temperature.

At a scanning rate of 4 K h^{-1}, a sample of crystalline *ortho*-terphenyl, kindly provided by S. S. Chang of the National Bureau of Standards, was found to melt at a temperature of 56.1 °C, 0.1 K less than the value obtained for this same sample of material using an adiabatic calorimeter[5]. In deducing this melting point, the temperature of transition was taken to be that at which the measured thermopile voltage began to deviate from its steady-state or baseline value, and a small correction (0.2 K) was applied due to the fact that the temperature-indicating thermocouple is not located directly in the sample vessel. For most biopolymer transitions, knowledge of the temperature of transition to within a few tenths of a degree should be satisfactory.

The half-response time of the instrument (thermopile voltage as a function of time) is about 50 sec. The peak area of the voltage–time thermogram obtained during a transition will be virtually unaffected by the lag due to the response time of the instrument.

The scanning rate of the instrument is adjustable up to 16 K h^{-1}. Faster rates would be possible if a power supply capable of supplying higher voltages were employed. The thermopile noise level of the loaded instrument, at scanning rates up to 15 K h^{-1}, is typically about 30 nV peak-to-peak.

The single thermopile offset resulting from steady-state heating of the copper block is a large signal and is approximately 8 mV at a scanning rate of 16 K h^{-1} with the cell filled with 1.6 cm^3 of water. In practice, it is desirable to compensate this rather large signal to utilize the more sensitive ranges of the amplifier to detect small thermal transitions which typically are deviations of only a few microvolts from these steady-state voltages. While the mode of operation commonly employed has been to connect the pairs of thermoelectric modules in opposition to compensate these large voltages, it is not possible to achieve a perfect matching of the two thermopiles. Hence, there is a resultant baseline which is not perfectly linear and possesses some slight curvature over a wide temperature range. In practice, it was found that filling one cell with the solution to be investigated and leaving the other empty, gave a very nearly linear baseline while scanning over the entire temperature range of the instrument. The absolute reproducibility of the baseline between separate runs using the same quantities of solutions is within a few tenths of a microvolt.

The instrument is extremely simple to operate, requiring only the activation of the power supply, control system, and recorder. Its operation is automatic until the run is ended and the various components turned off. The O-ring seals have never failed in over one hundred runs performed with the instrument. The instrument has been operated over the temperature range 5 to 90 °C.

The kind of results obtainable with this instrument will be illustrated by examples of two transitions. Figure 4 shows the gel-to-liquid crystal transformation of dipalmitoyl L-α-lecithin. As is seen, the transition is a fairly sharp one, occurring, in this case, over a temperature range of three degrees. The results of measurements made on the same loading of sample at various scanning rates are presented in Table 1. We are unable to discern any trend on the energy of transition with variation of the

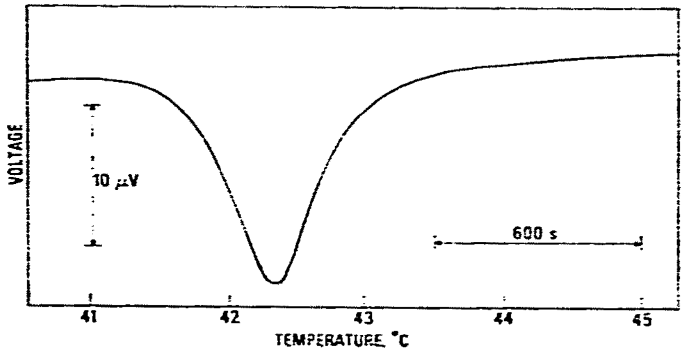

Fig. 4. Gel to liquid crystal transformation of dipalmitoyl L-α-lecithin. Scanning rate, 9 K h^{-1}.

TABLE I

THE ENERGY OF THE GEL-LIQUID CRYSTAL TRANSITION OF DIPALMITOYL L-α-LECITHIN IN WATER

Concentration is 1.371 mg lecithin per g water. Calorimeter contains 1.564 g of solution. The sample was obtained from Applied Sciences Laboratory. Relative molecular mass = 734.1. Average = -90.0_4 mJ. Estimated standard deviation of the mean = 0.47 mJ. $\Delta U = 30.8_3 \pm 0.32$ kJ mol^{-1}.

Run No.	*Scanning rate* $(K\,h^{-1})$	*Measured heat (mJ)*
1	4	-89.1_3
2	4	-89.8_5
3	9	-91.1_3
4	15	-89.1_9
5	15	-90.9_2

scanning rate. The energy of transition, 30.8 ± 0.3 kJ mol^{-1}, is in agreement with the value reported by Hinz and Sturtevant[6].

Data on a more diffuse transition, that of the poly(adenylic acid)-poly(uridylic acid) [poly (rA·rU)] helical complex unwinding to single strands of poly rA and poly rU over an approximately nine degree temperature range is shown in Fig. 5 and the results are presented in Table 2. A value of 32.0 ± 1.0 kJ per mole of base pair is obtained, again in agreement with previously reported measurements[7].

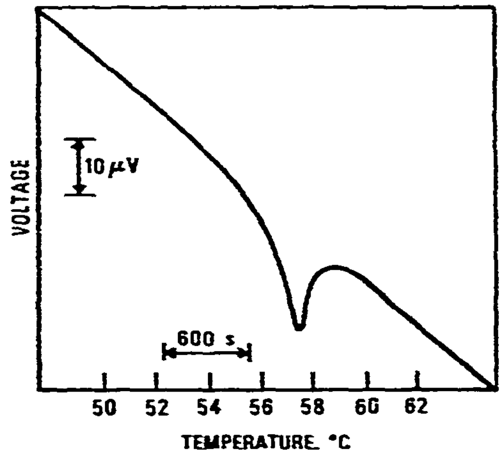

Fig. 5. Helical complex unwinding of poly (rA·rU) to poly rA and poly rU. Scanning rate, 16 K h^{-1}.

TABLE 2

ENERGY OF TRANSITION FOR poly (rA·rU) = poly rA + poly rU IN 0.9 M NaCl, pH 7.0

Scanning rate is 16 K h^{-1}. The poly (rA·rU) is from Miles Laboratories. The number of μmoles of base pair undergoing transition is based on analysis of phosphorus in the sample. Estimated standard deviation of the mean = 0.5 kJ (mol of base pair)$^{-1}$. ΔU = 32.0 ± 1.0 kJ (mol of base pair)$^{-1}$.

Run No.	*poly (rA·rU) (μmol)*	*Measured heat (mJ)*	*ΔU (kJ(mol of base pair)$^{-1}$)*
1	7.93	−247.2	31.2
2	7.93	−267.3	33.7
3	7.09	−218.9	30.9
4	7.09	−227.0	32.0
5	8.05	−258.7	32.1

Current work on protein solutions with this instrument indicates that a similar degree of imprecision (3%) is obtained for transitions occurring over a four degree temperature range and involving about 25 mJ of heat.

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