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BATCH-INJECTION ATTACHMENT FOR THE HART DSC *

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ABSTRACT

An attachment for performing batch injections or incremental titrations with a Hart Scientific model 7707 DSC is described. The effects of the attachment on calorimeter performance in the isothermal mode are minimal. The attachment can be used for determining heats of reaction under some circumstances, but is best suited for modifying the environment in the ampule around heat-producing samples such as living materials.

INTRODUCTION

The model 7707 differential heat conduction scanning calorimeter manufactured by Hart Scientific, Inc., can be effectively operated in an isothermal mode by simply scanning against the hardware limit. Although the baseline noise of about $\pm$ 3 μW is significantly larger than that obtainable with the non-scanning heat conduction calorimeters manufactured by Hart Scientific, Tronac and LKB ($\pm$0.1 μW), there are many measurements that can be done adequately and more conveniently and rapidly with the DSC operating in the isothermal mode. In comparison with other calorimeters, the multiple, removable ampules and relatively short settling time (20–30 min) of the Hart DSC make it particularly amenable for measurements on living biological materials that must be kept sterile and which do not have a long period of viability in which to make the measurement. For example, we have made many measurements of the metabolic heat rates of plant tissues and microorganisms under variable conditions of temperature, medium composition, and atmosphere [1–3]. Changing the medium or the atmosphere on the tissues during such an experiment, however, involves opening the calorime-

* Dedicated to Professor James J. Christensen in memory of his contribution to innovation in calorimetry.

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ter, removing and opening the ampules, changing the solution or gas in the ampules, closing and reinserting the ampules, and closing the calorimeter. Because of the thermal effects of these operations, the subsequent measurement requires the full settling time before useful data can be obtained. In order to increase the rate of measurements which scan composition, we have designed, built and tested an attachment which can be used to modify or change the liquid or gas phase in the sample ampules without opening the calorimeter. This injection device can be placed on or removed from the calorimeter in just a few seconds without the use of tools and requires no modification of the commercial instrument as presently produced by the manufacturer.

Two types of application are envisaged for the injection attachment. One is the use of the attachment to change the environment around a sample which subsequently changes its rate of heat production in response to the altered environment. In this case, the immediate heat of reaction of the titrant with the titrate is usually not of interest. In the second type of experiment, it is the heat of reaction of titrant with titrate that is of interest. An example of the first type of application is measurement of the effect of a change in ethylene or CO_2 concentration on the metabolic rate of a plant tissue sample. An example of the second type of application is the measurement of the heat of reaction of an acid with a base or the heat of solution of a solid in a liquid. In the first type of application, precise thermal equilibration of the titrant is not necessary because a small error in the temperature of the titrant only causes a slight delay before the subsequent measurement of heat rate can be made accurately. The titrant temperature must be controlled precisely in the second application, however, because it is the integral of the heat effect produced by the titrant addition that is to be determined.

DESCRIPTION OF INJECTION ATTACHMENT

The device consists of three parts connected together with flexible tubing. The first part is an ampule lid with three pieces of stainless steel needle tubing (0.8 mm o.d.) swaged through. One of the tubes extends just to the inside surface of the lid and is used to vent the ampule gases as needed. The other two tubes extend to within 0.1 mm of the bottom of the ampule. One of these tubes is used to add or remove liquids from the ampule and the other is used to add gases beneath the liquid level. The latter tube can be used to change the atmosphere over the sample and, in addition, serves the purpose of mixing and stirring the liquid. The two lids which are used to close the measuring-cell chamber have three slightly oversized holes drilled in them to accomodate the steel tubes and to allow these lids to move freely up and down and seat into the circular holes which they cover.

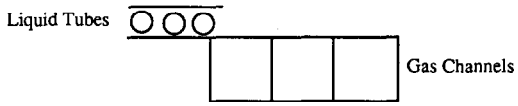

Fig. 1. Cross-sectional schematic diagram of the fluid equilibration ring of the injection attachment.

The second part of the assembly is a metal ring which sits on top of the constant-temperature block cover of the calorimeter. This composite ring contains machined channels for gas flow and circular lengths of stainless steel tubing for liquid flow. The purpose of this ring is to allow equilibration of the gases and liquids to the calorimeter temperature before addition to the ampules. A cross-sectional diagram of the ring is shown in Fig. 1 and a photograph of the entire ring is shown in Fig. 2. Each of the channels in the ring has a barrier which forces gas to flow completely around the ring before exiting to the ampule. Three sets of channels and tubes are provided in the ring, one of each for each of the three sample ampules in the calorimeter.

The third part of the assembly is a lid that fits snugly over the calorimeter measuring unit. This lid has an extended, square access trunk in the center which is covered with a smaller lid. The smaller lid and trunk give access to the ampules, connecting plastic tubes, and the ring. The flexible plastic tubing connects the various parts together within the access trunk. A shelf constructed around the top of the access trunk is used for mounting

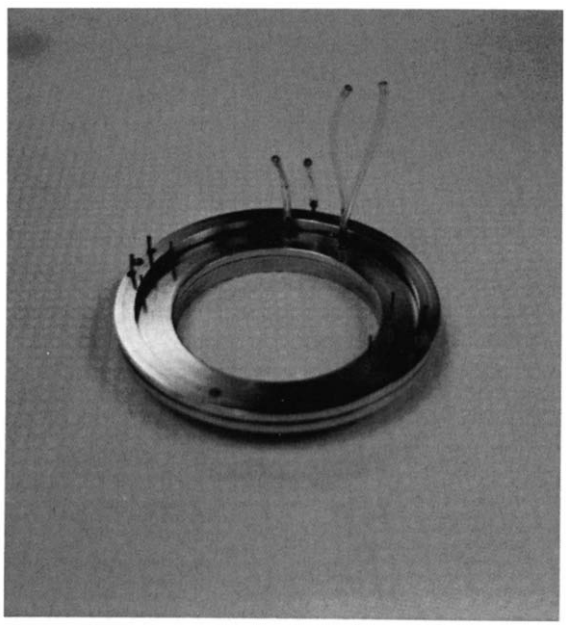

Fig. 2. Photograph of the fluid equilibration ring of the titration assembly.

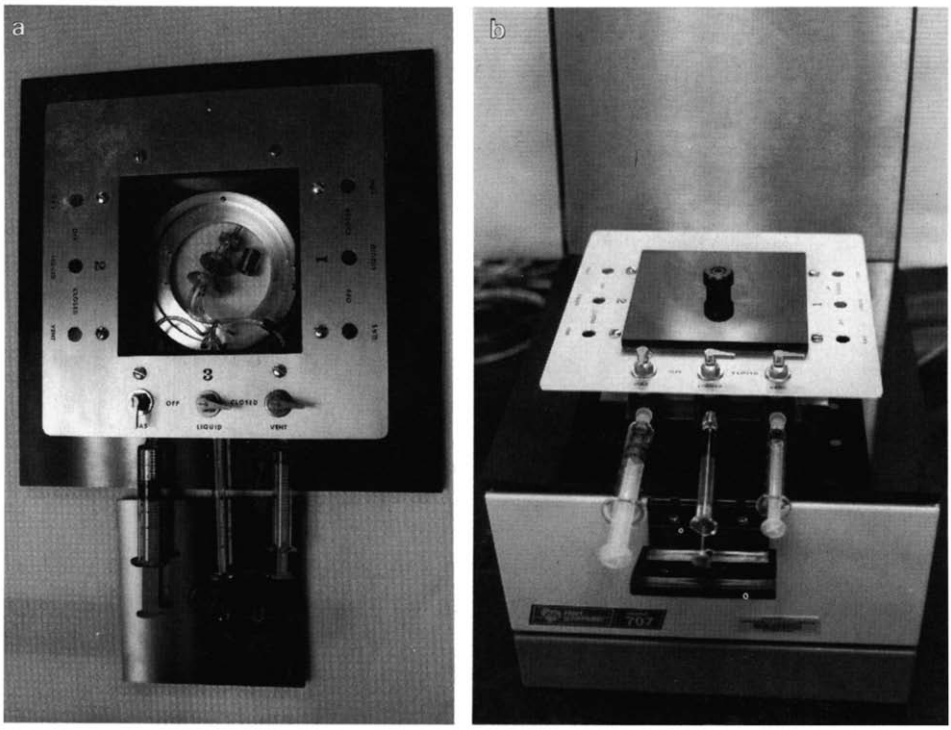

Fig. 3. (a) Photograph of the complete titration assembly. (b) Photograph of the titration assembly in place on the calorimeter. For clarity only one set of valves and syringes and one ampule were assembled to the unit for the photograph.

Hamilton valves and syringes which are used to control the addition and removal of materials from the ampules. Photographs of the lid assembled with the other parts and assembled on the calorimeter are shown in Fig. 3.

Tygon tubing was used to connect the parts together. No Teflon was used inside the access trunk because of the problem of possible contamination of the interior of the calorimeter by Teflon which has a large, thermally active phase transition near room temperature. During assembly and disassembly of the tubing there is a risk of abrading the tubing and thus dropping small shreds of plastic inside the measuring unit of the calorimeter. If Teflon were used, such contamination would require the calorimeter to be returned to the manufacturer for disassembly, cleaning, reassembly, and recalibration of the measuring unit before it could be used for temperature scanning.

To load the ampules, the assembly is simply lifted from the calorimeter and placed on a U-shaped rack. The ampules are left dangling from the tubing, and can readily be screwed onto or unscrewed from the lids by use of a split rubber ring to grasp the ampule lid and a rubber cup to grasp the ampule. Once the syringes are filled and the ampules are screwed into place, the entire assembly is simply lifted back onto the calorimeter and the

ampules are seated into the measuring cells by grasping the lengths of steel tubing protruding through the two measuring-cell lids with tweezers and gently moving the ampules and lids into place. After the ampules are in place, the trunk access cover is placed on top of the assembly.

EXPERIMENTAL

The calorimeter was electrically calibrated with (i) empty, unmodified ampules, (ii) with empty, modified ampules, and (iii) with modified ampules containing 0.5 ml of water. Baseline noise and position were also determined during the calibration experiments. A series of 100-μl injections of water were made into an initially empty ampule to determine the efficacy of thermal equilibration of the titrant to the calorimeter temperature. The reaction of 100 μl of 0.1 M HCl titrant with 500 μl of 0.18 M tris(hydroxymethyl)aminomethane (THAM) was used to test the feasibility of mixing by gas injection. In these experiments the injection of the HCl was followed by a series of 0.5 ml injections of air. Application of the injection attachment to the determination of the effect of environment on subsequent reactions is illustrated by some of our results obtained in a study of the effects of temperature and atmosphere composition on cauliflower metabolism.

RESULTS AND DISCUSSION

Because the heat conduction paths between the ampules and the surroundings are altered by the physical connections added to the lid, the baseline is significantly shifted when the injection assembly is used. The amount of the baseline shift is dependent on the temperature difference between the ampules and the room. The baseline shift is equivalent to 25 μW per °C difference between the calorimeter temperature and room temperature. The calibration constant of the instrument is altered slightly because of the additional heat loss paths through the lid (+1.2%), but is not significantly altered by the presence of water in the ampule. Since the new heat-loss paths are constant, the instrument can still be satisfactorily calibrated; the calibration constant is just different from that obtained with an unmodified ampule in place. With the titration assembly in place, short term baseline noise was unchanged, but long term noise in the baseline was found to be significantly increased and to have the same pattern and frequency as fluctuations in room temperature. This latter effect was eliminated by placing an insulating box over the calorimeter measuring unit.

The experiments in which water was injected into an empty ampule or into a partially filled ampule showed that the temperature of the equilibration ring was strongly influenced by the room temperature. A difference of

4° C between the calorimeter block temperature and the room temperature resulted in nearly a 1° C error in the titrant temperature as calculated from the heat effect resulting from the H_2O injection. Thus, significant differences between the titrant temperature and the calorimeter temperature will exist in the present design unless the room and the calorimeter are controlled to be the same temperature. Because of this difficulty and because Hart Scientific Co. has begun work on a new design of an injection attachment with a thermostatted equilibration ring, no further work on the determination of heats of reaction was pursued with the existing attachment. The water injection experiments made it clear, however, that the determination of heats of reaction of liquids is possible within an accuracy of about $\pm$ 5 mJ if the present attachment is used in a carefully thermostatted room. Heats of reactions of gaseous titrants could accurately be done with the present design without thermostatting the room.

In the injections of HCl(aq) into THAM(aq), approximately 90% of the heat of reaction is liberated during the injection of HCl. The remaining 10% is liberated during the first air injection. No significant amount of heat compared with the heat of reaction is liberated or absorbed by subsequent injections. Thus, one injection of 0.5 ml of gas is sufficient for complete mixing of easily mixed fluids. Subsequent injections of gas can be used to correct for evaporative heat effects which occur during the mixing injection. (Hart Scientific is currently considering the addition of an electric-motor driven stirrer to future injection attachment designs.)

Return of the output signal to baseline after an injection which produces or absorbs a significant amount of heat requires about 15 min or less. The settling time of the calorimeter with similar samples in unmodified ampules is about the same.

Figure 4 illustrates the most successful use of the present injection attachment, i.e. changing the environment around a biological sample. Using the injection attachment allows rapid observations of short term effects that are not observable if the ampule must be removed to change the environment inside the ampule. The first 40 min in Fig. 4 show the output as the sample and ampule equilibrate to the calorimeter temperature. The characteristic response of cauliflower metabolism to placement in a sealed container is exhibited between 40 and 125 min, i.e. an increasing rate followed by a decreasing rate. If the sample is left undisturbed the rate continues to decrease according to a first order rate law. Flushing the sample container with air, as was done at 125 min, causes the rate to increase again for a period of time before the decrease sets in again. Flushing with five volumes of Ar (at 175 min) is not sufficient to remove all the oxygen and the response cycle repeats again between 175 and 230 min. A second injection of five volumes of Ar depletes the oxygen in the ampule, and results in a rapid loss of metabolic activity as seen between 230 and 333 min. Based on the data in Fig. 4, we have postulated the existence of a gas-phase metabolic

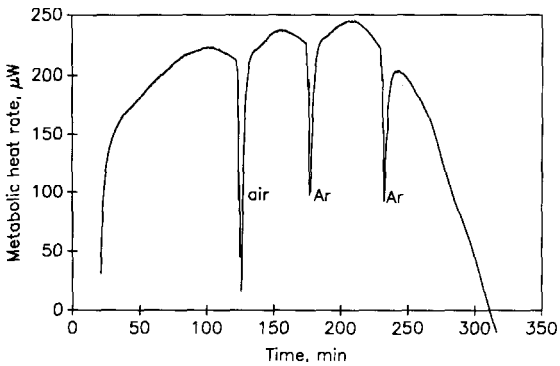

Fig. 4. Metabolic heat rate at 25 °C of a 268 mg cauliflower floweret as a function of time and atmosphere over the sample. The endothermic spikes at 125, 175, and 230 min correspond to 5 ml injections of air, Ar, and Ar, respectively, into the 1 ml ampule. These spikes result from the evaporation of water from the ampule which contained a 50 μl drop of water in addition to the floweret. The baseline for these data is at approximately −100 μW. A positive heat rate is exothermic.

modifier which enhances the metabolic rate at low concentrations and inhibits it at high concentrations. The data in Fig. 4 illustrate the usefulness of the injection attachment for the study of environmental effects on biological samples.

CONCLUSION

The Hart DSC can readily be modified to do batch and incremental injections by the addition of the attachment described in this paper. The attachment is of use when the calorimeter is operated in the isothermal mode. When the attachment is used for injecting high heat capacity fluids in situations which require measurement of the integrated heat effect, the room must be thermostatted at the same temperature as the calorimeter in order to avoid significant errors in matching the titrant temperature to the ampule temperature. The attachment is particularly useful for altering the environment around samples for which the heat rate is to be measured as a function of environment.

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