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A LIQUID EXPANSION MICROCALORIMETER

R. David[*] *and I. W. Hunter*

BioInstrumentation Lab, Department of Mechanical Engineering, Massachusetts Institute of Technology
77 Massachusetts Ave. 3-147, Cambridge MA 02139, USA

A novel microcalorimeter based on a miniature liquid-in-glass thermometer is described. Heat is transduced into an optical, rather than electrical, signal, facilitating a future array format. The instrument performs batch analysis (drop mixing) with a 2 μL sample volume. Energy changes of 4 μJ produced by a dilution of sulfuric acid are resolvable. The effect of evaporation, and measures taken to limit it, are discussed.

Keywords: *calorimeter, interferometer, liquid expansion, microjoule*

Introduction

Over the past few decades, calorimeters have been miniaturized and their resolutions improved in order to meet the needs of biochemical research and drug discovery. In recent years, several such instruments have been designed for potential parallelization for high throughput applications.

For a parallelized calorimeter, batch analysis (drop mixing) is more practical than flow injection analysis. Batch analysis microcalorimeters described in the recent literature have usually employed thin film thermopile temperature sensors [1]. In one design, mixing was performed by a syringe, with 100 μJ resolution in a sample volume of approximately 7 μL [2]. In another instrument, enzyme was immobilized on a thin film thermopile and 1 μL of analyte was introduced via a syringe pump, with apparently similar resolution [3]. Evaporation was cited as an important noise source for both these devices.

A thermopile-based device with sub-nanoliter sample volume has been constructed, and used to measure heats of evaporation of single drops [4]. A commercial arrayed calorimeter with projected well volume of 10–20 μL and resolution below 3 μJ is under development [5]. Finally, an array of twin calorimeters based on thermistor temperature sensors was fabricated [6]. Measurements with 0.5 μJ resolution were reported for individual reaction sites with 0.5 μL sample volume, with mixing performed by inducing electrostatic attraction between the drops.

In this paper, we describe a novel batch analysis microcalorimeter in which heat was transduced into an optical, rather than electrical, signal. This was accomplished by a liquid expansion temperature sensor with sub-microKelvin resolution [7, 8]. The optical signal mimicked labeling assays used for high throughput drug screening. It promises to allow future parallel measurements to be made without sacrificing resolution due to increased bandwidth, as in electrical devices.

We will also discuss measurement repeatability and describe strategies for coping with evaporation, two issues that are important in any batch analysis microcalorimeter design.

Experimental

The microcalorimeter's temperature sensor was a miniature liquid-in-glass thermometer with the meniscus level read by a Michelson interferometer. The expanding liquid in the thermometer was 6 μL of water. The sensor's measured resolution was better than any known thermometer above cryogenic temperatures [8]. A full description of the thermometer is available in [7, 8].

Figure 1 shows a schematic of the drop mixing setup. Two drops were placed side-by-side on top of the thermometer with a pipettor. The drops each had 1 μL volume, and were approximately 1 mm apart. Teflon tape wrapped around the glass surface of the thermometer provided a disposable, reproducible, and hydrophobic surface for the drops to sit on. One of the drops was a solution of sulfuric acid (Sigma-Aldrich) in distilled water, and the other was distilled water. Enthalpies of dilution for sulfuric acid in water were available in the literature [9].

The drops were physically mixed by a plastic pipette tip that was melted shut at its end. The pipette tip was mounted as a pendulum from an arm extend-

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[*] Author for correspondence: robertd@alum.mit.edu

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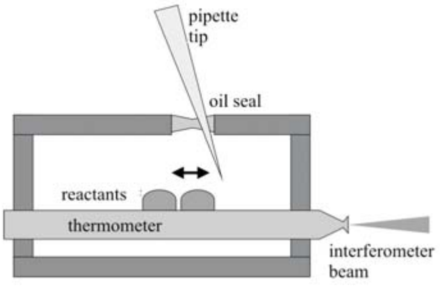

Fig. 1 Schematic cross-section of mixing setup

ing from the shaft of a stepping motor. This configuration minimized the vibration disturbance caused by the motion of the motor.

As noted by other researchers [2, 3], evaporation is a major obstacle in a batch analysis microcalorimeter. This is due to the large heat of vaporization (4.5 J in our case) contained in the reaction drops. (By comparison, the total surface energy was approximately 0.5 μJ.) Evaporation is slowed by the reduction in surface area upon mixing, and heat released by a reaction can also be lost to evaporation without entering the thermal sensor.

Several steps were taken to minimize evaporation. The reaction drops were sealed by mineral oil inside a chamber, seen in Fig. 1. The volume of water needed to saturate the air in the chamber surrounding the drops was only about 10 nL. Thus, condensation on surrounding surfaces was a greater concern. The pipette tip was hydrophobic, and all other surfaces were covered in either Teflon tape or oil to discourage condensation. A piece of membrane filter (Millipore HAWP 025 00) soaked in water was placed inside the chamber under the thermometer [2]. The large surface area of the water droplets in the filter produced a higher equilibrium vapor pressure than that of free drops.

The apparatus was temperature controlled and mounted inside a large enclosure constructed from vacuum insulation panels (Advantek Vaculok). Approximately seven hours were typically required for temperature equilibration after placement of the drops. Temperature control was ramped downwards. This kept the drops, which were thermally closer to the controllers, cooler than surrounding surfaces, as a further measure to reduce evaporation.

Results and discussion

Data for a control reaction of water with water are shown in Fig. 2. This trace was used as a reference and subtracted from the remaining data. The subtracted data for three acid dilutions, and two other controls, are shown in Fig. 3. Some experiments, in which the oil seal of the chamber broke, are not shown.

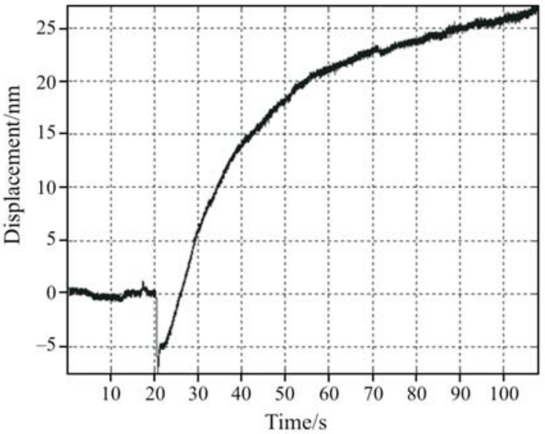

Fig. 2 Data from control reaction (water+water). The drops were mixed at 20 s. The data were detrended over the first 20 s

Heat loss from the thermometer was estimated using a solved problem for a rod geometry available in the literature [10]. The result is shown in Fig. 4. The temperature dynamics were similar to the experimental signal. At the peak of the response, 60% of the energy released in the reaction was predicted to be present in the thermometer.

Using this modeled result, together with the total heat capacity of the drops and thermometer (0.070 J K^{-1}), and the approximate sensitivity of the thermometer (0.22 mm K^{-1}), the expected response of the calorimeter was estimated. These estimates were in reasonable agreement with the measured values, as seen in Table 1. This indicated that an insignificant amount of heat generated by the reaction was lost to evaporation. In contrast, in experiments with no

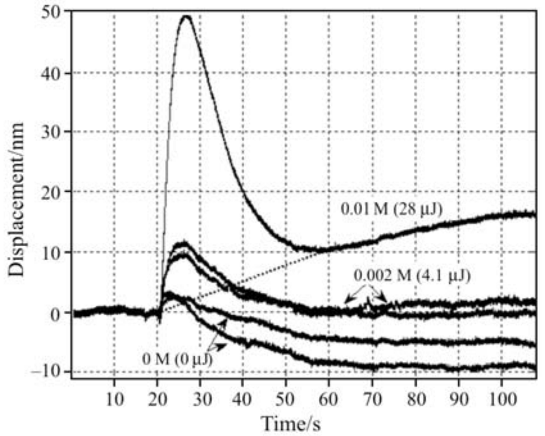

Fig. 3 Data from sulfuric acid dilutions, after subtracting the control trace in Fig. 2. The labels show the initial concentrations of sulfuric acid. The sizes of the peaks were measured between 20 and 60 s, as demonstrated for the 0.01 M peak by the dotted line

Table 1 Comparison of experimental with predicted results for the calorimeter response, as measured by the area under the peak (nm s)

Acid concentration	0.01 M	0.002 M	0 M
Experiment	712	139, 163	38, 8
Calculation	935	137	0

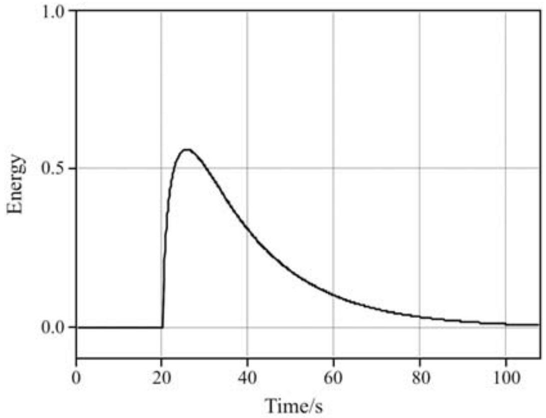

Fig. 4 Modeled energy content of the thermometer, as a fraction of the total released by the reaction

membrane filter near the drops, the response was half as large (not shown).

The sample volume was measured after each experiment and was typically 1.5 μL. Much of the missing 0.5 μL evaporated during the placement and alignment of the drops. The enthalpies of reaction were calculated accounting for the resulting higher concentrations of sulfuric acid.

The increasing temperature after mixing (seen in Fig. 2) was due to slower evaporation of the merged drop, which had lower surface area than the two original drops. The cooling spike seen immediately after mixing was attributed to evaporation induced by the motion of the pipette tip. Such a spike was seen even when the drops did not merge. Both these phenomena highlighted the fact that repeatability of the data depended on reproducible placement and mixing of the drops. This, rather than the temperature resolution of the thermometer, limited the energy resolution of the measurements. One possible avenue for eliminating disturbances arising from evaporation in a batch analysis calorimeter would be to measure the temperature of the entire chamber in which the drops rest.

Future work on our instrument includes minimization of the heat capacity of the thermometer, perhaps by replacing water with a gas as the expanding fluid. This will allow fuller advantage to be taken of the thermometer's very high resolution. Finally, differential (and arrayed) measurements will reduce common mode noise and decrease the time needed for temperature equilibration.

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