Micro-differential scanning calorimeter for liquid biological samples

Shuyu Wang, Shifeng Yu, Michael S. Siedler, Peter M. Ihnat, Dana I. Filoti, Ming Lu, Lei Zuo

Submitted to the Review of Scientific Instruments

October 2016

Center for Functional Nanomaterials
Brookhaven National Laboratory

U.S. Department of Energy
USDOE Office of Science (SC),
Basic Energy Sciences (SC-22)

Notice: This manuscript has been authored by employees of Brookhaven Science Associates, LLC under Contract No. DE- SC0012704 with the U.S. Department of Energy. The publisher by accepting the manuscript for publication acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes.
DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or any third party’s use or the results of such use of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof or its contractors or subcontractors. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.
Micro-differential scanning calorimeter for liquid biological samples

Shuyu Wang¹, Shifeng Yu², Michael S. Siedler³, Peter M. Ihnat⁴, Dana I. Filoti⁴, Ming Lu⁵, Lei Zuo²*

¹ Department of Mechanical Engineering, Stony Brook University, Stony Brook, NY 11794, USA
² Department of Mechanical Engineering, Virginia Tech, Blacksburg, VA, 24061, USA
³ AbbVie, Deutschland, 67061 Ludwigshafen, Germany
⁴ AbbVie Bioresearch Center, Worcester, MA 01605, USA
⁵ Center for Functional Nanomaterials, Brookhaven National Laboratory, Upton, NY 11973, USA

*corresponding author: leizuo@vt.edu

Abstract: We developed an ultrasensitive micro-DSC (differential scanning calorimeter) for liquid protein samples characterization. This design integrated vanadium oxide thermistors and flexible polymer substrates with microfluidics chambers to achieve high sensitivity (6V/W), low thermal conductivity (0.7 mW/K), high power resolutions (40nW) and well-defined liquid volume (1µL) calorimeter sensor in a compact and cost-effective way. We further demonstrated the performance of the sensor with lysozyme unfolding. The measured transition temperature and enthalpy change were in accordance with the previous literature data. This micro-DSC could potentially raise the prospect of high-throughput biochemical measurement by parallel operation with miniaturized sample consumption.

Keywords: microcalorimeter, differential scanning calorimeter, protein unfolding, flexible substrate, microfluidics, MEMS (micro electro-mechanical systems)

Introduction:

Kinetic processes are playing a critical role in physical transformations, such as glass transition, and in macromolecule transformations, such as protein denaturation. Understanding the effects of external influences on the transformation may provide valuable insights into the process[1]. The first step to such understanding is to detect the reaction accurately in the real time. Calorimetry is the technique that has been widely used to study such kinetic process and thermodynamic properties of the materials or biological system[2]. Among several different operation modes of calorimetry, differential scanning calorimetry (DSC) is a well-known dynamic calorimetric method. It imposes a temperature ramp continuously and measures heat capacity change of the material (both in liquid and solid forms).

For the past decades, the micro/nanofabrication technology has been enabling calorimeters to be smaller, faster and more accurate. As a result, the conventional bench-top scale instruments were miniaturized into microelectromechanical sensor systems and some may integrate microfluidics systems [3-5]. These miniaturized calorimeters were often called micro/nanocalorimeter or chip calorimeters. Normally, they were based on the thin films, either made of silicon/silicon nitride [6, 7] or polymers [5, 8]. These chip calorimeters were capable of measuring samples with very small thermal mass due to significantly reduced addenda[9] and may measure the energy on the
order of nanojoule[4] accounted from the superb thermal insulation. We have already seen chip calorimeters’ application to study thin films [8], polymer [10], cells [11], protein [12] and so on.

Such studies in biological systems can be very crucial to the pharmaceutical industry as several critical steps in the drug discovery process are associated with calorimeters. Currently, the long measurement time of the conventional scanning calorimeters often requires years to finish thousands of the chemical compounds[13] and the liquid volume of protein sample required is very large(500µL-2mL). Chip calorimeter might be a potential solution since they use very small amount of protein sample and can easily provide parallel operations to achieve high-throughput[12]. However, chip calorimeter as DSC to study biological system still remains very scarce in academics compare to micro isothermal titration calorimeter (ITC) [4, 12, 14, 15] due to technology difficulties to develop them[13].

Several different configurations of micro-DSC for liquid sample appeared in recent years. Olson [2] and Youssef[16] studied the scanning calorimeter with liquid samples, yet their uses are limited to measure latent heat of transformation due to evaporation. Yao [17] built an AC calorimeter with glass capillary tube that consumed 10µL liquid. The device (heat capacity resolution ±300nJ/K) successfully tested the lysozyme solution’s heat capacity when denaturing. Garden’s [18] scanning AC calorimeter has also measured 5µL biological liquid sample’s heat capacity with high resolution(±150nJ/K). They used a gasket and polyimide membranes (supported by micro-posts) to construct the chamber. However, their design suffered from large thermal conduction (30mW/K) which limited the sensitivity. Lee[19] constructed an enclosed parylene chamber (1µL) to tested DNA and hydrocarbon yet the signal-to-noise ratio is too low. Lin’s group successfully integrated microfluidic systems in the DSC using polydimethylsiloxane (PDMS) microfluidics chamber (1.2µL) and SU8 diaphragm to and reached high detection limit (30nW)[20]. They further developed AC-DSC[5] and changed substrates to flexible polyimide substrates. These devices reached very high performance (sensitivity 4-8V/W) when demonstrating with protein samples[21]. Yet, the thermopiles deposited on the flexible substrate could not determine absolute temperature, and therefore the transition temperature might not be accurately measured.

In this paper, we reported the fabrication and operation of a micro-DSC for liquid biological samples which can address the above problems. We showed how to make a flexible calorimeter by microscale fabrication technology and integrate it with PDMS microfluidics chamber (1µL). Due to the highly temperature-sensitive vanadium oxide thermistors and superb thermal insulation of polyimide thin films, the device had proven to be very sensitive to thermal events. This enabled the sensor to be used for protein interaction detection at low cost and disposable way. This technology could readily scale up to array format to apply in high-throughput drug discovery.

**Theory**

Virtually all DSC measures the temperature difference between the sample and reference region since this temperature difference can be converted proportionally to heat flow rate difference based on a simple linear model. This conversion is the zeroth approximation, and more accurate conversions can use higher-order approximations[22].

Within one chamber, the differential heat equation can be described as Eq.1, where $C_p$ is the heat capacity, $G$ is the heat conduction, and $P$ is power or heat flow.

$$C_p \frac{dT}{dt} + G(T(t)-T_0) = P(t)$$ (1)

The time constant $\tau$ is determined by $\frac{C_p}{G}$. When time constant is much smaller than the biomolecular thermal event (time constant of guanidine induced unfolding is on the order of
(20-50s[17]), the process can be considered as in the steady state. Therefore, $\Delta T = T_s - T_r$ can be related to $\Delta P = P_s - P_r$ with a linear relationship $\Delta P = G \Delta T$. Using temperature sensors to generate voltage signal $\Delta U$ proportional to the $\Delta T$, we can have

$$\Delta U = S \Delta P$$

(2)

where $S$ is the sensitivity of the calorimeter. Consequently, the thermal insulation of the chamber ($G$) and temperature sensor’s sensitivity can determine the device’s sensitivity. Based on the mathematical model, the heat capacity difference between the sample and reference solution $\Delta C = C_s - C_r$ can be obtained from Eq3 where $\dot{T}$ is the scanning rate of the calorimeter.

$$\Delta C = \frac{\Delta P}{\dot{T}}$$

(3)

Since the differentiation method used in the DSC measurement, the symmetry of the sample and reference can be crucial to the final results.

**Device description and fabrication**

Figure 1 and Figure 2 showed the 3D schematic view and the cross section view of the microcalorimeter respectively. The device consisted of two parts: the PDMS microfluidic chamber and the flexible calorimeter sensor. They were fabricated separately and later bonded together to formulate the whole device. There were two identical microfluidic chambers: one for protein liquid sample and the other for reference buffer liquid sample. The chamber had a volume of 1$\mu$L (height 200$\mu$m, radius 1.25mm) and was surrounded by air gaps to reduce the thermal mass of the close chamber and increase the thermal insulation. We cast the liquid PDMS onto the master pattern made from SU8 100 photoresist and then solidified it by soft baking. Last, we drilled the tiny holes for inlet and outlet of the chambers. The microfluidic chamber could serve to prevent liquid evaporation and provide well-defined liquid volume.

The flexible calorimeter sensor used polyimide substrate (7$\mu$m) and integrates the thermistors and the microheaters for sensing and calibration. ANSYS simulation was used to assist the pattern design of the microheater: the widths of the heater in each circle were finely tuned to reach the maximum temperature uniformity while heating. The Au traces (width and gap were 10$\mu$m) were the metallization features and served for electrical interconnection of the thermistor layer. This design was amount to parallel connection of multiple resistors and could significantly lower the resistance of the thermistor.

We spun coat PI 2611(HD microsystem) polyimide on a silicon wafer and ramping annealed it from room temperature to 350°C to make it chemical inert. The solidified polymer layer would have very low adhesion with the silicon wafer but enough to bond them together during fabrication so that easy separation of them could be achieved (Figure 3(b)). We fabricated the Au microheaters (Figure 3(c)), the polyimide dielectric layer, sputtered vanadium oxide thermistors, Au traces for thermistor (Figure 3(d)), and the polyimide moisture protection layer sequentially. The 100nm vanadium oxide layer was deposited by DC sputter and Au trace was deposited by e-beam evaporation. In order to enhance the bonding strength of the flexible sensor and microfluidics chamber, a thin layer of PDMS diluted with toluene would be spun coated before peeling off from the silicon substrate. After oxygen plasma treatment for 30s under 20mTorr, the microfluidic chamber and flexible sensor would be bonded together. Figure 3 (a) showed the fabricated device after bonding.
Experimental methods

The electrical circuit connection was illustrated in Figure 4. The two thermistors for sample and reference liquid and two outside variable resistors were connected as a Wheatstone bridge in order to provide common mode rejection. Such bridge circuit could pick up the temperature difference between the sample and reference side. The voltage output of the bridge circuit represented the temperature difference between the sample and reference chamber. We used the lock-in amplifier (Stanford SR830) to provide the voltage input \( V_+ \) and differentiate the voltage output from the bridge \( V_{out} \). The sourcemeter (Keithley 2636) was the power source for the microheaters and measures the resistance change during the thermal response test. To calibrate the resistance of the thermistors and do temperature scanning, we placed the microDSC inside a high temperature probe station and further connect the wire outside to the equipment. This high temperature probe station could raise the temperature with a program and provide the thermal shielding at the same time. We used lysozyme samples (molar weight is 14307 Da) at a concentration of 100 mg/mL to demonstrate the device’s performance. The buffer used was 10...
mM HEPES pH 7.4 and 150 mM NaCl. The whole experimental measurement and control were achieved with LabVIEW programs.

![Figure 4. Schematic view of the micro-DSC circuit connection. The thermistors and outside variable resistors form a Wheatstone bridge.](image)

**Results and discussions**

Before doing the measurement, we first calibrated the vanadium oxide thermistors. The resistance and temperature’s relation is plotted in log scale (Figure 5). The thermistor’s resistance is normally in the range of 15-40KΩ, meaning the designed gold trace pattern can effectively reduce the resistance to an appropriate range. The temperature coefficient of resistance (TCR) can be obtained from the slope of LnR versus temperature as -2.8%/°C, which is one order higher than a traditional platinum thermistor. We also observed good uniformity of the thermistor material; the sample and reference thermistors could have 1-2% variation in resistance. Although the thermistors could introduce self-heating during measurement, the thermistor on the reference side can counterbalance it, and such self-heat can be further neglected during temperature scanning. Unlike the thermopiles that require large numbers of them to gain high sensitivity, the high-performance thermistors can achieve compactness with simplified fabrication[23]. Moreover, thermistors can detect the absolute temperature within the small chamber, which is necessary for DSC measurement.

![Figure 5. Temperature calibration of a thermistor and vanadium oxide resistivity measurement as a function of temperature](image)

We further calibrated the microcalorimeter using electric power applied to the microheater. The thermal response to a step function power can be predicted by $T(t) = \frac{P(t)}{G}(1 - e^{-t/\tau})$. By fitting curve of data in Figure 6 to exponential increase function, we could extract the thermal time $\tau$ constant to be 6 s. Due to the well-designed air gap and the high thermal insulation of PDMS, the affected addenda thermal mass was minimized to be much smaller than the liquid samples especially when small amount of heat was applied. Therefore, the thermal conductance ($G = \frac{C}{\tau}$) could be calculated to be around 0.7mW/K. Such low thermal conduction could be comparable to the open-type calorimeters [12, 24].
The performance about noise level of the device was also evaluated. We filled the two chambers with deionized water and tested it at a constant temperature in a well-defined environment. Due to the inevitable asymmetry of the two sides, the measured voltage signals might drift. Figure 8 was the result after subtracting the raw data from three order fit of the data. The rms noise at 1 Hz bandwidth is 250nV, corresponding to 40nW of power resolution and 60µK of temperature resolution (the results are calculated based on the sensitivity S and thermal conduction value G). This noisy behavior could be related to the environment temperature fluctuation control, the extent of the common mode rejection and the thermistors’ Johnson noise.

We demonstrated the micro-DSC’s performance with the lysozyme protein (100mg/mL) for consecutively two times. We raised the temperature of the chamber from 25°C to 80°C at the rate of 7.5°C /min. After baseline subtraction, the measured voltage outputs from the Wheatstone bridge showed the typical DSC curve of protein denaturation. We could further obtain the partial specific heat capacity of lysozyme as a function of temperature (Figure 9) based on Eq3. The melting temperature were at around 61°C. These results were further integrated to obtain the enthalpy change (ΔH) of the lysozyme samples (Figure 10). The values obtained in this
study were around 415KJ/mol, which corresponded well with the published data 377-439 KJ/mol [25]. Apart from these, we could see the second test could almost repeat the first time test. Such variation of the results might be related to the difference in the protein samples.

Figure 9. micro-DSC’s two tests with lysozyme sample (molar weight:14307 Da). Specific heat capacity of lysozyme vs. Temperature. (baseline subtracted)

Figure 10 enthalpy change of two lysozyme samples vs. Temperature during unfolding. The enthalpy is obtained by integrating the data in figure 9.

Conclusion

This paper reported a micro-DSC applied for liquid biological sample characterization. This sensor implemented temperature sensitive vanadium oxide as the thermistor material with TCR value of -2.8%/°C. With the help of the high thermal insulation provided by the thin polyimide membrane and the well-designed microfluidics chamber, a sensitivity of 6V/W and power resolution of 40nW were achieved. This high-performance micro-DSC successfully tested the lysozyme unfolding with the minimum sample consumption (1µL) and this technology could be further applied to expand the devices into arrays so that high throughput measurement in the drug discovery industry might be attained.

Acknowledgment

The authors thank the funding support from NSF (IDBR #1530508) and AbbVie Inc. The fabrication was carried out at the Center for Functional Nanomaterials (CFN) at Brookhaven National Laboratory, which is supported by the U.S. Department of Energy, Office of Basic Energy Sciences, under Contract No. DE-AC02-98CH10886. We also thank Dr. Fernando Camino and Kuan Hu for the help and useful discussion.

Reference


