A MEMS ISOTHERMAL TITRATION BIOCALORIMETER

Bin Wang, Yuan Jia, Qiao Lin
Columbia University, USA

ABSTRACT

We present a MEMS-based isothermal titration calorimeter (ITC) for characterization of biomolecular interactions. This device consists of thermally isolated reaction microchambers, chaotic micromixers, and a thin-film thermopile. We present experimental results from using the device for ITC measurements of the biochemical reaction of 18-Crown-6 (18-C-6) and barium chloride ($BaCl_2$), as well as the ligand-protein binding of cytidine 2'-monophosphate (2'CMP) and ribonuclease A (RNase A), in a 1-μL volume at concentrations as low as 2 mM. Consistent temperature-dependent thermodynamic properties are also obtained, demonstrating the potential of the device for characterization of biomolecular interactions with minimized reagent consumption.

KEYWORDS

MEMS, isothermal titration calorimetry, biomolecular interaction, binding isotherm, differential calorimetry.

INTRODUCTION

Isothermal titration calorimetry (ITC) directly probes thermodynamics of biomolecular interactions by detection of heat evolved as a function of the molar ratio of reactants. It can simultaneously determine all binding parameters with a single set of experiments, and thus provides an efficient, high-precision, label-free method for characterization of biomolecular interactions. ITC is widely used in various applications such as fundamental scientific investigations, drug discovery and biotherapeutic development. However, conventional ITC instruments [1] have been limited by complicated construction, slow thermal response, and large reagent consumption. While MEMS technology holds the potential to address these issues [2], its application to ITC has been scarce [3]. Current MEMS-based ITC approaches typically involve continuous flow-through channels or droplet generation and manipulation. Flow-through ITC measurements are conducted typically without well-defined volumes and thus difficult to obtain quantitative information associated with the reaction, while droplet-based ITC measurements can be significantly affected by energy dissipation via evaporation. In addition, the existing MEMS calorimetric devices generally lack mixing capabilities, which is critical for reaction between microfluidic samples, and are generally difficult to accurately control the environment temperature for temperature-dependent studies of biomolecular interactions. Here, we present a MEMS-ITC device that integrates thermally isolated calorimetric chambers, on-chip microfluidic mixing, and sensitive thermoelectric detection. This device allows MEMS-based ITC measurements with well defined miniature reaction volumes and at properly controlled temperatures, potentially enabling efficient thermodynamic characterization of biomolecular interactions.

EXPERIMENT

In an ITC measurement, a binding reagent is titrated in known aliquots into a sample, while the reaction heat is measured and used to calculate the thermodynamic properties, including the equilibrium binding constant (K_B), stoichiometry (N), and enthalpy change (ΔH). Our MEMS-ITC device (Fig. 1) integrates two identical PDMS microchambers (each 1 μL) situated on a freestanding polyimide diaphragm and surrounded by air-cavities for effective thermal isolation. The chambers are integrated with an antimony-bismuth thermopile and connected to the inlets through a passive chaotic micromixer. The mixer uses herringbone-shaped ridges in the ceiling of a serpentine channel to generate a chaotic flow pattern that induces mixing of the incoming liquid streams [4]. For ITC measurements, the sample and binding reagent introduced into the device are first mixed in the mixer, and then enter the sample calorimetric chamber. Meanwhile, the sample and pure buffer are also introduced, becoming mixed before entering the reference calorimetric chamber. The differential temperature between the chambers is measured using the integrated thermopile, and is used to compute the thermal power from the reaction, and in turn, the thermodynamic parameters.

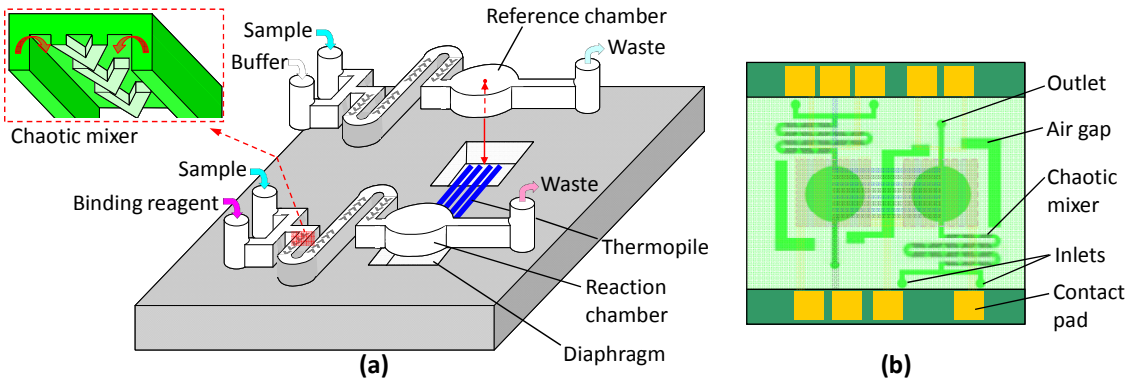


Fig. 1. (a) MEMS ITC design and (b) top view of the device schematic.

The MEMS ITC device was fabricated using micromachining and soft lithography techniques. The microfabricated device integrated a 50-junction Sb-Bi thermopile and two 1-µL calorimetric chambers. During ITC measurements, the device (Fig. 2) was placed in a custom-built low-noise, temperature-controlled enclosure where the thermopile output was measured. The sample and ligand were introduced using syringe pumps.

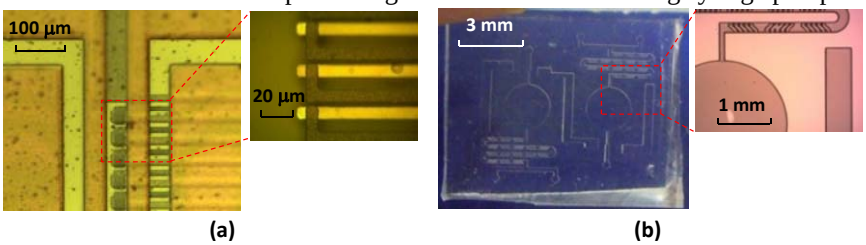


Fig. 2. Images of important chip elements on (a) the thermal substrate and (b) the PDMS structure.

Our calibration experiments indicated that the device had a thermal time constant of 1.5 s with a linear steady-state thermal response (responsivity: 4.9 mV/mW) (Figs. 3a and b). We also calibrated the device's sensitivity at controlled temperatures (provided by the thermal enclosure) from 20 to 45°C, and found it remained almost unchanged with a relative variation of less than 3%. The device was used for ITC measurements of a model biochemical reaction system consisting of 18-Crown-6 (18-C-6) and barium chloride ($BaCl_2$). The time-resolved device output exhibited a stable baseline throughout the measurements and a reaction-specific spike (Fig. 3c) upon introduction of 5 mM $BaCl_2$ and 4 mM 18-C-6 (each 0.5 µL) with no appreciable delay, indicating full mixing of the reactants. Also note that the reaction completed in approximately 20-30 s, during which interference from solution injection (shorter than 1 s) was generally negligible.

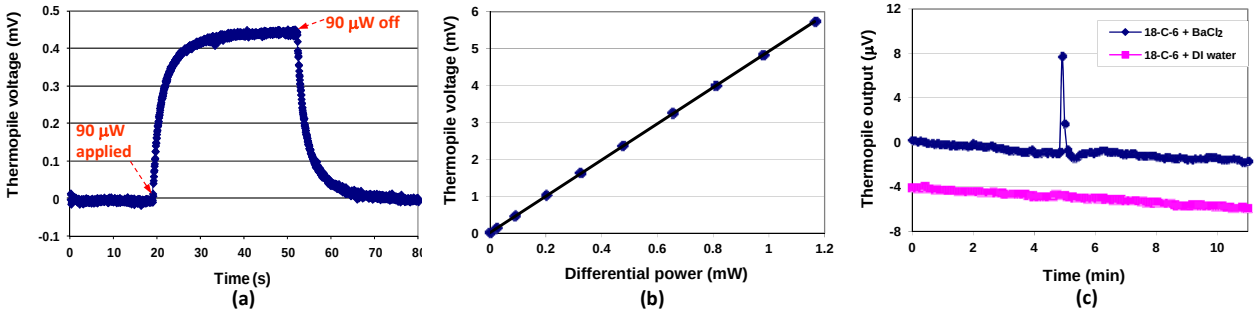
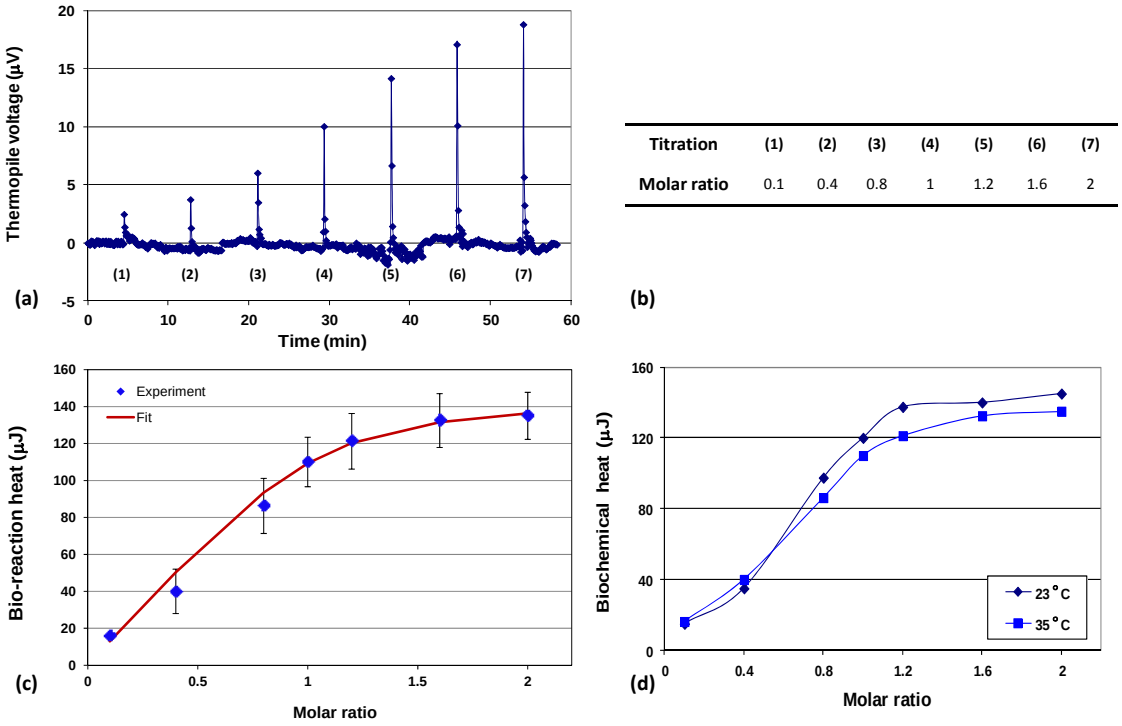


Fig. 3. Calibration of the MEMS ITC device's (a) transient and (b) steady-state response to differential thermal power; and (c) time-resolved output upon introduction of a model biochemical reaction system.



Titration	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Molar ratio	0.1	0.4	0.8	1	1.2	1.6	2

Fig. 4. (a) Device output (baseline subtracted) of the reaction of 5 mM 18-C-6 and $BaCl_2$ at a series of molar ratios shown in (b), (c) calculated biochemical heat of binding as a function of molar ratio, with fitted curve to an analytic model, and (d) binding isotherm of 18-C-6 and $BaCl_2$ at different temperatures.

The baseline-subtracted device output demonstrated spikes consistent with the titration reactions (Fig. 4a) in which the molar ratio ($BaCl_2$/18-C-6) was varied from 0.1 to 2 (Fig. 4b). Rather than measuring the heat evolved with the addition of several aliquots of $BaCl_2$ to a single sample of 18-C-6 as performed in commercial instruments, our ITC experiment was performed at discrete measurements [3] each with a definite molar ratio. The thermopile voltage was then used to calculate the thermal power and in turn, the reaction heat during the interaction. The reaction heat as a function of reactant molar ratio generated a binding isotherm [5], from which the thermodynamic properties can be obtained through fitting to an established analytical model [6] (Fig. 4c). Note that our device affords detectable sample concentrations approaching those of conventional instruments (ca. 1 mM) [1] with roughly three orders of magnitude reduction in volume. We performed ITC measurements of the reaction of 18-C-6 and $BaCl_2$ at controlled temperatures of 23 and 35 ºC (Fig. 4d), and used the resulting binding isotherms to compute the temperature-dependent thermodynamic properties of N, K_B and ΔH (Table 1). These properties and their temperature dependence obtained by our measurements agree reasonably with published data using commercial calorimeters [1].

Table 1. Temperature-dependent thermodynamic properties of the reaction of 18-C-6 and $BaCl_2$.

	Temperature (ºC)	N	K_B (M^{-1})	ΔH (kJ/mol)
MEMS-ITC data	23	1.00	~6.0×10^3	30.0
	35	1.05	~2.8×10^3	27.8
Published data	25	1.01	5.63×10^3	29.9
	40	0.97	3.17×10^3	29.4

We further applied the MEMS-ITC device for characterization of biomolecular interactions, e.g., ligand-protein binding, using a demonstrative system of cytidine 2'-monophosphate (2'CMP) and ribonuclease A (RNase A) [5]. Again, we performed measurements at a series of molar ratios (2'CMP/RNase A) with the evolved heat calculated at controlled temperatures of 23 and 35 ºC (Fig. 5). In turn, the temperature-dependent thermodynamic properties associated with this biomolecular interaction were determined (Table 2) and again found to agree reasonably well with published data using commercial ITC instruments [5]. These results demonstrate the potential utility of this MEMS-ITC device for efficient characterization of a wide variety of biomolecular interactions.

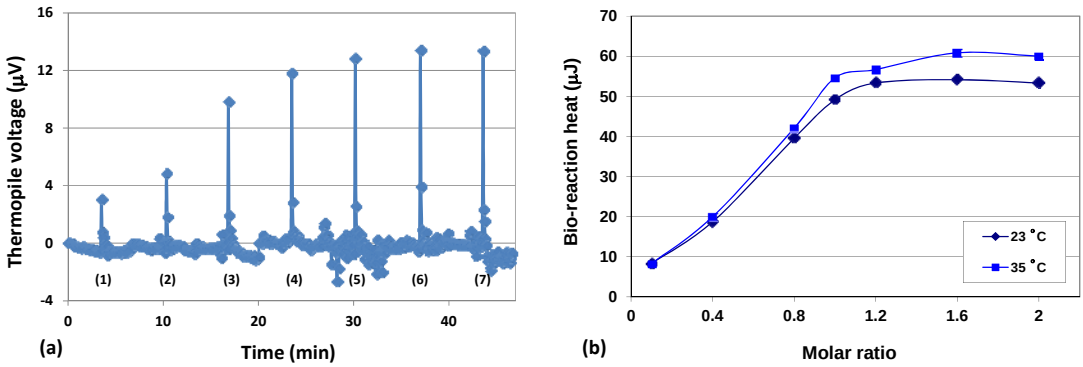


Fig. 5. Device output (baseline subtracted) of the binding of 2 mM RNase A and 2'CMP at a series of molar ratios, and (b) binding isotherm of RNase A and 2'CMP at different temperatures.

Table 2. Temperature-dependent thermodynamic properties of the binding of RNase A and 2'CMP.

	Temperature (ºC)	N	K_B (M^{-1})	ΔH (kJ/mol)
MEMS-ITC data	23	1.01	~9.0×10^4	52.3
	35	1.07	~4.0×10^4	56
Published data	28	1.00	8.27×10^4	51.4
	38	1.04	4.85×10^4	57.5

REFERENCES

[1] MicroCal LLC, www.microcal.com.

[2] W. Lee et al., *High-sensitivity microfluidic calorimeters for biological and chemical applications*, PNAS, vol. 106, pp. 15225-15230, (2009).

[3] M.I. Recht et al., *Enthalpy array analysis of enzymatic and binding reactions*, Analytical Biochemistry, vol. 377, pp. 33-39, (2008).

[4] A.D. Strook et al., *Chaotic mixer for microchannels*, Science, vol. 295, pp. 647-651, (2002).

[5] T. Wiseman et al., *Rapid Measurements of Binding Constants and Heats of Binding Using a New Titration Calorimeter*, Analytical Biochemistry, vol. 179, pp. 131-137, (1989).

[6] MicroCal LLC, *ITC Data Analysis in Origin: Tutorial Guide*, (2004).

CONTACT

Bin Wang 1-212-854-3221 or bw2176@columbia.edu