Dual-inlet micro-calorimeter for bio-chemical studies of microliter liquid samples

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Abstract

We present a fluidic micro-calorimeter that shows high versatility measuring both thermal properties of samples and enthalpies of bio-chemical processes. The micro-calorimeter consists of two stacked chips with silicon nitride membranes that define the liquid chamber of 2.6 μl. The chamber has two inlets which allow real time reactions monitoring. We measured the thermal conductivity and diffusivity of water-methanol solutions with 1% resolution, and we determined the mixing enthalpy for water-methanol system with a signal/ noise ratio of 20.

1. Introduction

Micro-calorimeters have great potential as bio-chemical sensors due to their capability to analyze small quantities of samples within short time at low cost. Most micro-calorimeters are used to investigate the enthalpy and kinetics parameters involved on biochemical processes by measuring the heat transfer during such processes [1, 2]. There is also great interest in using calorimetry to measure materials' thermal conductivity (k), diffusivity (α) and heat capacity, although, only a few studies demonstrate its application on microliter liquid samples [3, 4]. In this paper, we demonstrate a micro-calorimeter designed to determine all relevant thermal properties and additionally the enthalpy of bio or chemical processes on 2.6 μl liquid samples. Recently, we demonstrated the sensor capability to measure thermal diffusivity (α) and conductivity (k) of microliter samples [4] we present now a second generation sensor with two inlets instead of only one to explore its potential to measure enthalpy of reactions. As a proof of

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concept, we will study the micro-calorimeter performance by measuring thermal properties of aqueous solutions of methanol with several concentrations and by measuring the enthalpy of mixing methanol with water.

2. Experimental

2.1. Device description

The microfluidic calorimeter is composed of two stacked thermal sensor chips like schematically shown in Fig. 1(a). Both chips have a 2 μm-thick free-standing silicon nitride membrane with an integrated heater and thermopile. The membranes are separated by 300 μm and the spacing between them delimits the fluidic chamber with 2.6 μl volume. The heater is located in the center of the membrane and has a typical electrical resistance (R_h) of 19.3 kΩ. The thermopile has the cold junctions located at the rim of the silicon chip while the hot junctions are located on top of the membrane, 80 μm away from the heater, and follow the temperature changes inside the fluidic chamber. A picture of the chip calorimeter is shown in Fig. 1(b) [4]. Two inlets and one outlet are micro-machined into the bottom chip allowing the liquid samples to go in and out of the fluidic chamber and also the reagents to mix inside the chamber. The device is assembled on top of a customized aluminum manifold composed by a network of drilled fluidic channels which let the liquid samples flow in and out the calorimeter device without leakage.
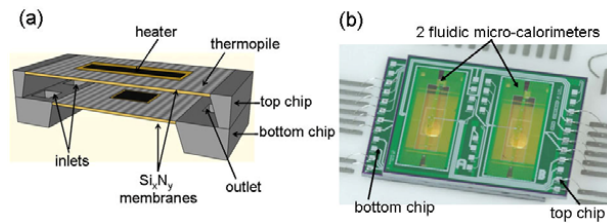

Fig. 1. (a) Schematic illustration of the dual-inlet fluidic micro-calorimeter chip first picture; (b) top view of the calorimeter.

2.2 Thermal properties measurements

Modulated calorimetry is used for the determination of k and α. An ac voltage is applied to the bottom membrane heater and, by Joule effect, an ac heat wave with a frequency twice the input frequency, f, is produced. A lock-in amplifier (Stanford Research Systems SR830) is used to measure the amplitude, V_{RMS}, and phase shift, θ, of the heat signal that reaches the top membrane thermopile. The measurements are all performed at 20ºC. The input signal amplitude is $V_0 = 5$ V. For thermal conductivity and thermal diffusivity determination input frequencies of 10 mHz and 1 Hz are used, respectively. At 10 mHz input signal, V_{RMS} can be described by[4]:

$$V_{RMS} = \frac{N\alpha_S V_0^2}{2R_h\left[\left(k_{liq} + k_{air}\right)\frac{A}{d}P_1 + K_{mem}P_2\right]} \quad (1)$$

Where: P_1 and P_2 - fitting parameters that indicate the model accuracy; $N\alpha_S$ - thermopile sensitivity (~ 40 mV/K); A – area of the heater (~ 0.6 mm²); d - distance between membranes (~ 0.3 mm); K_{mem} – thermal conductance of bottom membrane (~ 150 μW/K); k_{liq} – thermal conductivity of liquid sample; k_{air} – thermal conductivity of air (0.025 W/Km).

At 1Hz input signal, θ is given by:

$$\theta = -\sqrt{\frac{2\pi f}{4\alpha}}P_3 d - P_4\frac{\pi}{4} \quad (2)$$

A calibration of the device has to be done using liquids with know thermal properties in order to extract P_1, P_2, P_3 and P_4. For thermal properties measurement methanol-water solutions are prepared beforehand to make sure that

there is no influence of the heat of mixing two components on the output signal. Methanol, MeOH, is mixed with deionized (DI) water in concentration between 0.1 % and 100 % (v/v). The solutions are introduced through one of the inlets into the micro-calorimeter chamber and measurements are performed with no flow.

2.3 Mixing enthalpy measurements

Mixing methanol and water is an exothermic process. The enthalpy of mixing methanol and water is studied for methanol–water concentrations (v/v) between 0 and 50%. The reagents are pumped in the calorimeter chamber through separate inlets using a peristaltic pump (Chemyx Fusion 200) and using two syringes. The measurements are taken place in two stages. First, the reagents are pumped at 50 µl/ min into the calorimeter chamber. Then, the flow is stopped to allow completion of the mix. The temperature increase is measured by the top thermopile and the output voltage from the thermopile is acquired as a function of the time using a multimeter (Agilent 34970A).

3. Results

3.1 Thermal properties of methanol-water solutions

The micro-calorimeter shows a sensitivity of 5 V/W and thermal resistance of 200 K/W with the chamber filled with water. Figure 2(a) shows k of methanol-water mixtures as a function of methanol volume fraction, x_{MeOH}. The inset shows the calibration curve for the device used on these measurements. k can be reproducibly measured within ~0.3% error. k values obtained for x_{MeOH} = 0.2, 0.4, 0.6 and 0.8 are according with previous studies [5] within ~1.5 % deviation. Additionally, k of methanol-water mixtures decrease with methanol volume fraction, following the empiric Jordan correlation [6] for mixtures of two liquids. Figure 2(b) presents the α as a function of methanol volume fraction. The inset shows the thermal diffusivity calibration curve. α values are measured within ~0.3% error and the measured data shows a good agreement with literature data [5, 7] and the modified empirical Jordan correlation [7]. Figure 2(a) and 2(b) indicate that thermal properties can be determined for solution concentrations down to 1%(v/v) which is very close to the best resolution attained up to now by Matvienko *et al.* (0.5% v/v) using the pyroelectric thermal wave cavity technique that requires higher volumes of samples [7].
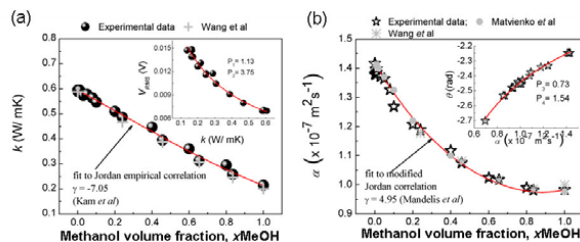

Fig. 2. (a) Thermal conductivity versus methanol concentration in water at 20ºC. (b) Thermal diffusivity versus methanol concentration in water at 20ºC.Both insets shows the calibration curve using several liquids of well known thermal conductivity at 20ºC.

3.2 Enthalpy of mixing methanol and water

Figure 3(a) shows the output signal when methanol and water are mixed on a 50% (v/v) proportion. When the pump is started, water and methanol start mixing inside the chamber. There is an initial output peak and then the signal stabilizes on ~56 mV. The signal/ noise ratio is 22. The flow rate is higher than the rate of mixing. Thus, only a percentage of the methanol and water molecules available in the chamber can mix. When the pumps are stopped, the methanol and water inside the chamber continue the mixing process until a maximum output signal of 75 mV is attained. Afterwards the signal decreases to about 0 V in 40 s. The enthalpy of mixing, ΔH_{mix} can be related with the area underneath the output signal: $\Delta H_{mix} n_{mix} = Q_{mix} = A_{mix} = A_1 + A_2$, where A_1 is the area underneath the output curve obtained at constant flow for the time interval that the reagents are inside the chamber (3.2s), A_2 is the area

underneath the output peak formed after stopping the pump, Q_{mix} is the heat produced during mixing and n_{mix} is the total number of moles in the mixture. In figure 3(b) it is presented the A_{mix} as a function of the expected heat produced in the calorimeter chamber, $Q_{mix,E}$. The inset shows the expected mixing enthalpy as a function of the methanol molar fraction. Literature enthalpy values are used [8]. It is assumed that the volume of mixed solution equals the chamber volume (2.6 µl). A linear fit to the data shows a slope, b=10, which indicates that the number of moles contributing to heat production is about 10 times lower than expected. Since the reaction chamber has a volume of only 2.6 µl, microfluidics physics laws are valid. The Reynolds number for water and methanol is 6 and 8, respectively, thus the system is restricted to the laminar flow regime, making the mixing of water and methanol difficult. The slope value shows that the effective volume of water and methanol that mix is about 10 times smaller than the volume of the reaction chamber.
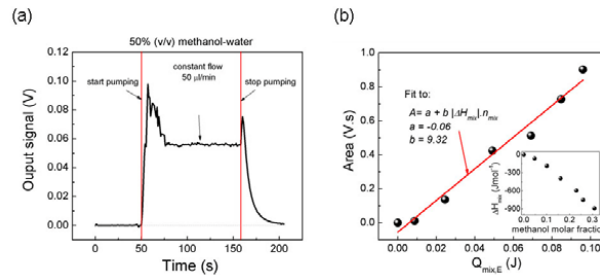

Fig. 3. (a) Output signal from thermopile resulting from mixing methanol with water at 50%(v/v) fraction at 20ºC. (b) Area underneath the output signal vs. enthalpy of mixing methanol-water with different molar fractions. The inset shows the enthalpy of mixing vs. methanol molar fraction.

4. Conclusion

We demonstrate a fluidic micro-calorimeter showing high resolution and high throughput on the analysis of microliter samples for lab-on-a-chip applications. Thermal properties and mixing enthalpies have been successfully measured for the methanol-water system. Thermal properties can be measure with a resolution of 0.1% methanol concentration (v/v) and 0.3 % reproducibility. The heat of mixing methanol and water is measured with a signal to noise ratio of 22. The design of the device is not optimized to have the maximum mixture of the two reagents and further improvements can be done in order to increase the diffusion processes.

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