

Chip Calorimeter for Thermal Characterization of Bio-Chemical Solutions

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Abstract

A chip calorimeter to measure thermal properties of bio-chemical fluid samples is successfully demonstrated. The chip calorimeter design enables the simultaneous measurement of thermal conductivity and thermal diffusivity of 2.5 μl liquid samples with high-sensitivity and high-throughput. The micro-calorimeter consists of two silicon nitride membranes, with integrated heaters and thermopiles that are 300 μm apart and enclose the liquid chamber. The sensor shows 5 V/W sensitivity and 200 K/W thermal resistance for water. The applicability of the micro-calorimeter to bio-chemical fluids thermal characterization is demonstrated by measuring thermal conductivity and thermal diffusivity of methanol-water mixtures and protein aqueous solutions (bovine serum albumin, BSA). Methanol-water mixtures thermal properties reveal good agreement with earlier reported experimental and theoretical data, showing approximately 2 % deviation. Thermal properties of BSA solutions with concentrations up to 20 % (w/v) were measured and values for thermal conductivity and diffusivity of (pure) BSA were estimated to be 0.20 W/Km and $0.64 \times 10^{-7} \text{ m}^2/\text{s}$, respectively. The fluidic chip-calorimeter resolves thermal properties for concentrations down to 1 % of both methanol and BSA.

1. Introduction

Measuring thermal properties of microliter liquid samples has showed increasing importance with the development of microfluidics and lab-on-a-chip applications [1-3]. Medical and biochemical applications require minimal sample consumption and highly sensitive sensors able to detect diluted samples. Calorimetry has been commonly used to measure thermal conductivity (k), thermal diffusivity (α) and heat capacity (C_p) of liquid samples [4-6], although few studies demonstrate its application on microliter volume capacities [7, 1, 8, 3]. Chip 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 of the chip calorimeters are limited to the measurement of one of the three thermal properties. Previous studies demonstrated the measurement of thermal conductivity or specific heat of liquid samples in either open or closed chamber configuration [9, 3]. In such cases the heater and thermopile are usually placed in the same membrane, only a few microns away from each other. Thus, the effective thermal diffusivity of the membrane-sample systems is dominated by the thermal diffusivity of the membrane. A

CMOS sensor was also proposed where diffusivity of liquid samples dropped directly onto the device is measured [10].

Recently, we proposed a fluidic chip calorimeter whose design allows the simultaneous measurement of both thermal conductivity and thermal diffusivity of microliter liquid samples [8]. Heat capacity can be further determined ($C_p = kV/\alpha$, where V is the volume of the cell). The main feature of such device is that the liquid sample is kept inside a chamber where heater and thermopile are only separated by the sample. Moreover, working with a closed chamber chip calorimeter allows the accurate characterization of volatile liquids.

In this paper we will present a second generation of the fluidic chip calorimeter that has two inlets (instead of one) which allow the mixture of reagents inside the chamber and real-time measurements. We will study the chip calorimeter performance and ability to measure thermal conductivity and thermal diffusivity of bio-chemical liquid samples by using two test systems: water-methanol mixtures and Bovine Serum Albumin (BSA) aqueous solutions. Thermal properties of water-methanol mixtures have been intensively studied using different techniques [11-14] allowing a good quality comparison and interpretation of the results obtained using the fluid chip calorimeter. Additionally, the study of protein solutions is motivated by the fundamental role that thermal properties, especially heat capacity, has on understanding folding processes and molecular recognition [15-16, 9]. One of the most common methods to study such proteins processes is differential scanning calorimeter (DSC) where the heat capacity is measured as a function of the temperature. However, DSC uses volume cells in the order of 1 ml which are not suitable for biochemical applications. This paper will also focus on determining thermal properties of BSA solutions with different concentrations at 20 °C and verify the minimum concentration that can be resolved.

2. Device description

The microfluidic calorimeter is composed of two stacked thermal sensor chips like schematically shown in Fig. 1. 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 2.5 μl volume. The heater is made of p-type polycrystalline silicon (polySi); it is located in the center of the membrane and has a typical electrical resistance (R_h) of 19.3 k Ω . The thermopile consists of 136 p-polySi/ n-polySi thermocouples. The cold junctions are located at the rim of the silicon chip where they are kept at room temperature. 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 showing the chip calorimeter is shown in Fig. 1b. The detailed fabrication process is described elsewhere [8].

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. 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. 1c).

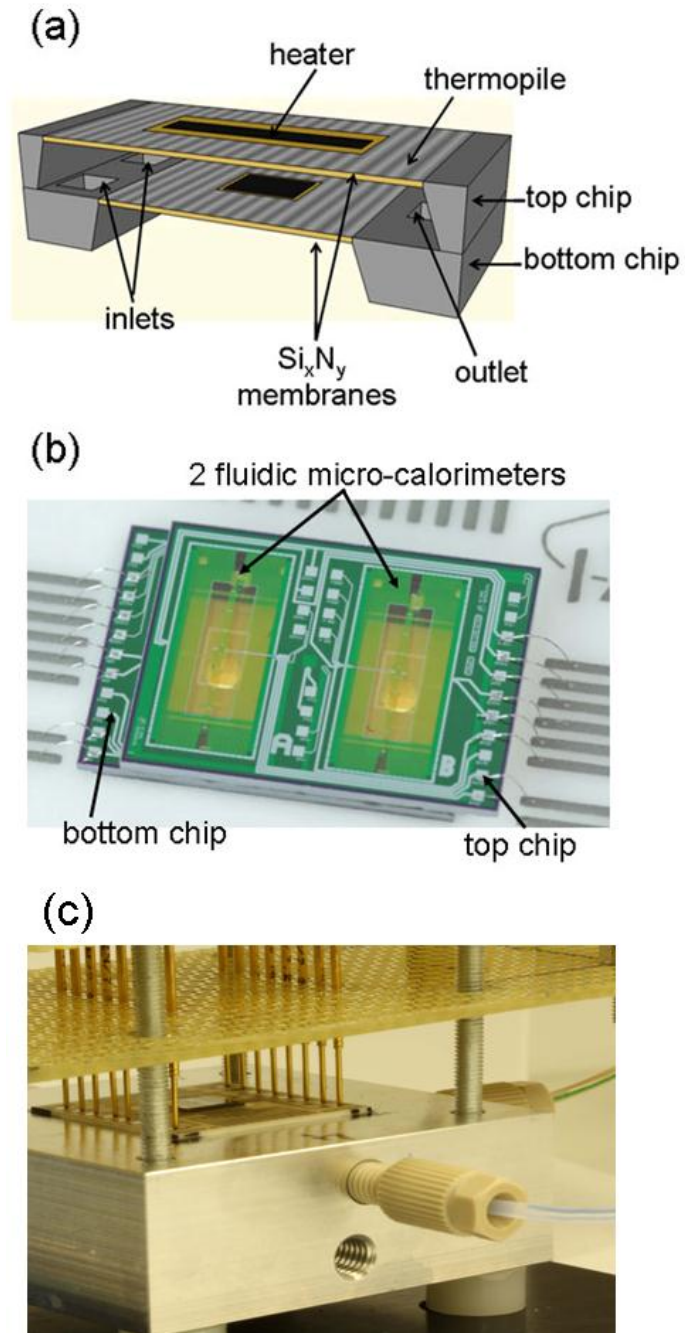


Fig. 1 Fluidic chip-calorimeter composed of two stacked chips with silicon nitride membranes that define the liquid chamber of $2.5 \mu\text{l}$. The micro-calorimeter has 2 inlets micro machined in the bottom chip. (a) Chip calorimeter side view schematics; (b) Top view image showing two calorimeters on one chip (c) Measurement set-up.

3. Measurement conditions

Modulated calorimetry is used for the determination of thermal conductivity and thermal diffusivity. An AC voltage with frequency f_0 is applied to the bottom membrane heater and by Joule effect an ac heat wave with a frequency, f , twice the input frequency is

produced. The heat wave propagates through the liquid sample resulting in amplitude decay and phase delay, and is detected by the thermopile placed on the opposite membrane. 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. For all the measurements the input signal amplitude is $V_0 = 5$ V. The measurements are all performed at 20 °C. According to [5], a generic expression (1) can be deduced that relates the change in temperature with the applied voltage and the angular frequency of the heat wave:

$$\Delta T = \frac{V_0^2}{2R_h} \left(\frac{1}{K} - \frac{1}{\omega C} \frac{\cos(\omega t - \theta)}{\sqrt{1 + (\omega C / K)^{-2}}} \right) \cong \frac{V_0^2}{2R_h K} (1 - \cos(\omega t - \theta)), \text{ when } \omega C / K \ll 1 \quad (1)$$

Where:

ΔT - difference in temperature measured by the thermopile between the chamber and silicon rim;

K - effective thermal conductance of the system;

C - effective heat capacity of the system;

V_0 - amplitude of input ac voltage;

R_h - heater resistance;

ω - angular frequency of initial ac voltage ($\omega = 2\pi f$).

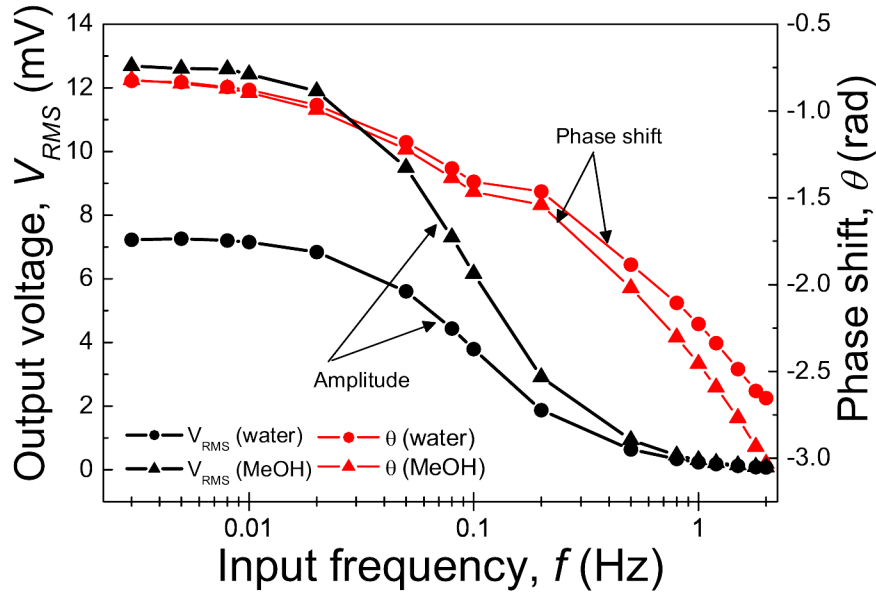


Fig. 2 Amplitude, V_{RMS} , and phase shift, θ , of thermopile output measured as a function of the input frequency, f , when the chamber is filled with water and methanol, @ 20°C.

Furthermore, the temperature difference is related to the voltage perceived in the thermopile according to $V = N\alpha_s\Delta T$, where α_s and N are respectively the Seebeck coefficient and the number of thermocouples.

In the limit of low frequencies, the change in temperature is independent of the frequency just as the amplitude of the signal detected by the thermopile.

$$V = \frac{N\alpha_s V_0^2}{2R_h K} \Leftrightarrow V_{RMS} = \frac{N\alpha_s V_0^2}{2\sqrt{2}R_h K} \quad (2)$$

This behavior is evidenced in Fig. 2 that shows the amplitude and phase shift of thermopile output measured as a function of the input frequency, f_0 , of the input signal when the chamber is filled with water and methanol. At very low frequencies, between 0.001 Hz and 0.01 Hz, V_{RMS} is approximately constant. It follows that Eq. (2) can be used for frequencies as high as 0.01 Hz. Considering the above observations, an AC voltage signal with 5 V amplitude and 0.01 Hz initial frequency is used for thermal conductivity measurements.

Fig. 2 illustrates also that while the output amplitude decreases with frequency, f_0 , the phase delay increases. A simplified expression is presented in [4] that relates the thermal diffusivity with phase delay of the heat wave using modulated calorimetry. For that, it is assumed that the heat diffusion occurs in one dimension (1D), and the sample thickness, d , is longer than the thermal diffusion length, l :

$$\theta = -\sqrt{\frac{2\omega}{2\alpha}} d - \frac{\pi}{4}, \quad \text{when } l = \left(\sqrt{\frac{2\omega}{2\alpha}}\right)^{-1} < d, \quad \omega = 2\pi f_0 \quad (3)$$

Approximating the calorimeter to a 1D system - the distance between membranes (300 μm) is smaller than the membranes width (2 mm) and length (2.8 mm) - the heat is mainly transferred through the liquid in the direction perpendicular to the silicon membranes. Eq. (3) will be valid for heating frequencies above 0.25 Hz. Thus, thermal diffusivity measurements are done applying to the heater a sinusoidal wave ($V_0 = 5$ V, $f_0 = 1$ Hz) and measuring the phase delay with the thermopile.

4. Characterization of methanol-water mixtures and BSA aqueous solutions

All 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, (Boom) is mixed with deionized (DI) water in concentration between 0.1 % and 100 % (v/v). Bovine serum albumin, BSA, (Sigma Aldrich) aqueous solutions were also prepared with DI water to obtain concentrations between 0.005 % and 20 % (w/v).

The solutions are introduced through one of the inlets into the micro-calorimeter chamber and the thermal conductivity and thermal diffusivity measurements are performed with no fluid flow. Each measurement is performed at least 3 times, inserting each time fresh solution into the calorimeter chamber. In the case of measuring BSA solutions the fluidic chamber is cleaned, between each measurement, by flushing it with methanol and DI water. Additionally, DI water is used as an experimental control by measuring its thermal properties after each time the chamber is cleaned.

5. Results and Discussion

Device calibration

A calibration of the micro-calorimeter is done to relate the output voltage of the thermopile with the liquid thermal properties. The voltage amplitude, V_{RMS} , and phase shift, θ , are measured and plotted against the values of thermal conductivity and diffusivity for reference liquids whose properties can be found in the literature [11-14] [17] (Fig. 3). The liquids used for calibration were: water, methanol, ethanol, iso-propanol, acetone, ethylene-glycol and propylene-glycol and 20%, 40%, 60% and 80% (v/v) methanol-water solutions. The calibration curve was built using more than one referenced value for each liquid measured. Concerning water, a comparison between previous thermal conductivity and diffusivity studies show deviations of nearly 1%. The micro-calorimeter under study shows a sensitivity of 5 V/W and thermal resistance of 200 K/W with water.

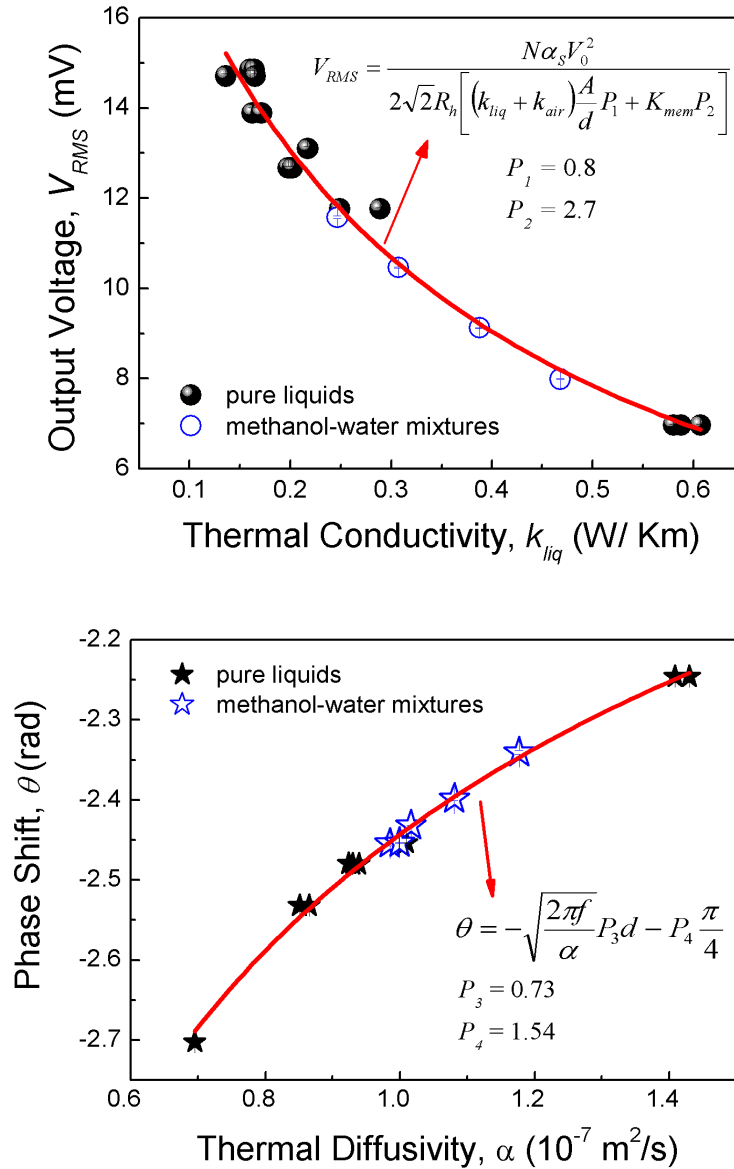


Fig. 3 Calibration curves for thermal conductivity (a) and thermal diffusivity (b) @ 20°C.

In Fig. 3a the measured signal amplitude is plotted as a function of the thermal conductivity of the calibration liquids using $f_0=0.01$ Hz input signal.

Experimental data is fitted to an expression based on (2):

$$V_{RMS} = \frac{N\alpha_S V_0^2}{2\sqrt{2}R_h \left[(k_{liq} + k_{air}) \frac{A}{d} P_1 + K_{mem} P_2 \right]} \quad (4)$$

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 - thickness of air and liquid layer – equivalent to the distance between membranes (~ 0.3 mm);

K_{mem} – thermal conductance of bottom membrane (~ 150 μ W/K);

k_{liq} – thermal conductivity of liquid sample;

k_{air} – thermal conductivity of air (0.025 W/Km).

Eq. (4) considers that heat is transferred from the heater on the bottom membrane to: i) the liquid sample, ii) air below the bottom freestanding membrane and iii) to the membrane itself. The model is based on the assumption that the heat transferred to the liquid and air flows only in the direction perpendicular to the membranes (d), across the area of the heater (A). In the membrane the heat is assumed to flow in the direction of the silicon rim. The total thermal conductance of the system is given by: $K = K_{liq} + K_{air} + K_{mem}$, where K_{liq} and K_{air} , are the thermal conductances of the liquid and air. Considering that the volume of air under the bottom membrane is equal to the volume of liquid on top of it (as depicted in Fig. 1a), $K = (k_{liq} + k_{air}) A/d + K_{mem}$. The experimental data fits well with Eq. (4). The fitting parameter $P_1 = 0.8$ is very close to unity which means that the value theoretically calculated for A/d is a good estimate. However, $P_2 = 2.7$, indicates that the heat transferred through the membrane is underestimated. The simplified model shows nevertheless a good agreement with the real system calorimeter-liquid sample.

Fig. 3b shows the measured phase shift for 1 Hz input signal as a function of the thermal diffusivity of the calibration liquids. The measured data fit well to Eq. (5):

$$\theta = -\sqrt{\frac{2\pi f_0}{\alpha}} P_3 d - P_4 \frac{\pi}{4} \quad (5)$$

where $P_3 = 0.73$ and $P_4 = 1.54$ are fitting parameters that indicate the model accuracy. The data fitting shows that the 1D model used (Eq. 5) is a good approximation to the calorimeter system.

Methanol-Water mixtures

Figure 4 shows the thermal conductivity and thermal diffusivity of methanol-water mixtures as a function of methanol volume fraction, x_{MeOH} . Both, k , and α can be

reproducibly measured within $\sim 0.3\%$ error. The k and α values obtained for $x_{MeOH} = 0.2, 0.4, 0.6$ and 0.8 are according with previous studies [11, 13-14] within $\sim 2\%$ deviation. Additionally, the plot shows that k for methanol-water mixtures decreases with methanol volume fraction, following the empiric Jordan correlation [18] for mixtures of two liquids:

$$k = k_{water}^{x_{water}} k_{MeOH}^{x_{MeOH}} \left\{ \exp(\gamma [k_{water} - 3k_{MeOH}]) \right\}^{x_{water} x_{MeOH}}, \text{ where } \gamma \text{ is an empirical constant.}$$

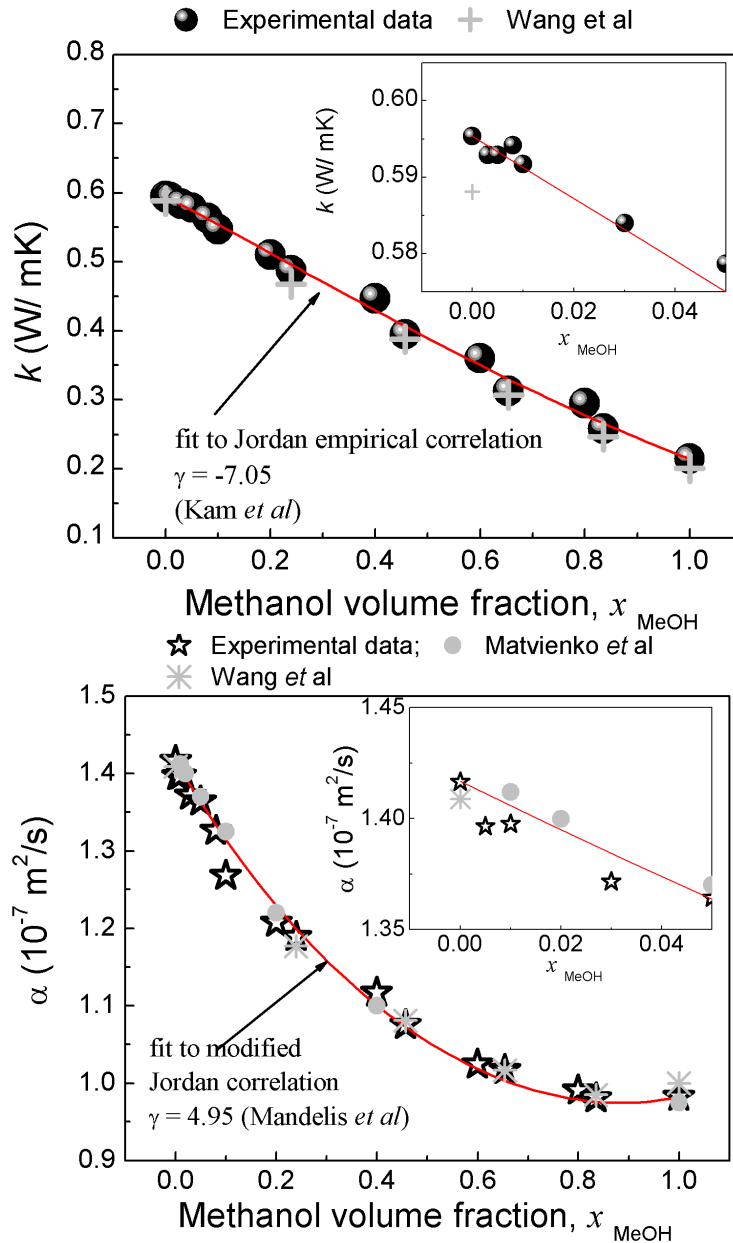


Fig. 4 Thermal conductivity and thermal diffusivity vs. methanol volume fraction in methanol-water solutions @ 20 °C.

Experimental data of α for methanol-water mixtures show a good fit with a modified Jordan correlation which is based on the relation between k and α ($\alpha=k/\rho c$, where ρ and c are the mixture density and specific heat)[13]:

$$\alpha = \frac{k_{water}^{x_{water}} k_{MeOH}^{x_{MeOH}} \left\{ \exp(\gamma [k_{water} - 3k_{MeOH}]) \right\}^{x_{water} x_{MeOH}}}{x_{water} \frac{k_{water}}{\alpha_{water}} + x_{MeOH} \frac{k_{MeOH}}{\alpha_{MeOH}}}$$

The insets of figure 4a) and 4b) show in detailed the experimental points measured for solutions with methanol concentrations lower than 5%. It is observed that the solutions thermal conductivity and diffusivity can only be distinguished from pure water for methanol volume concentrations $\geq 1\%$ ($x_{MeOH} = 0.01$). The maximum resolution reported for thermal diffusivity measurements of methanol-water mixtures is 0.5% using the pyroelectric thermal wave cavity technique which requires higher volumes of samples [13].

Bovine Serum Albumin aqueous solution

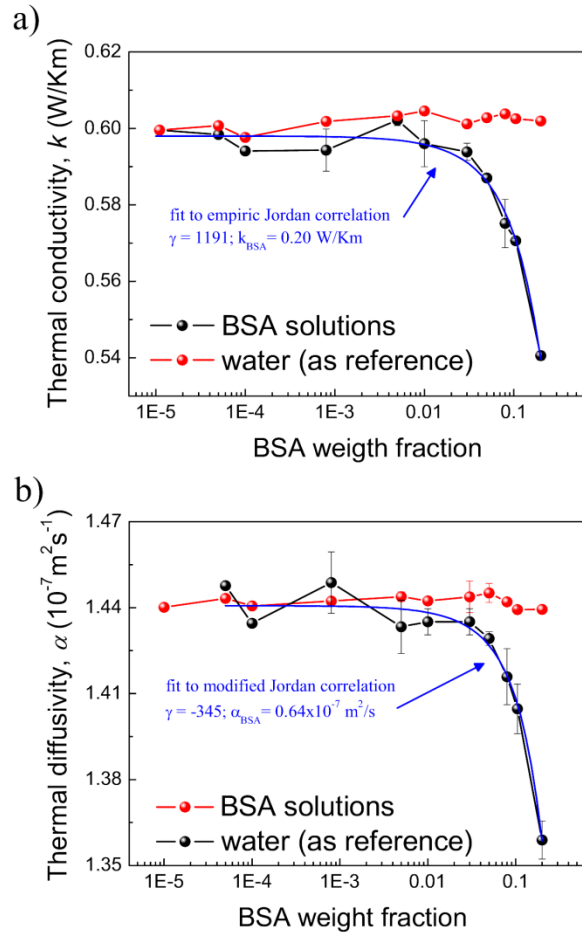


Fig. 5 Thermal conductivity (a) and thermal diffusivity (b) vs. BSA concentration in aqueous solutions @ 20 °C.

Fig. 5a) and 5b) show the thermal conductivity and thermal diffusivity measured, respectively, as a function of BSA weight fraction. Similarly to methanol-water mixtures the sensor resolves k and α down to 1 % BSA concentrations. The measurement error can reach 1.5 % which is substantially higher than the one observed for methanol-water mixtures. Such a high value could be caused by such artifacts as: i) small air bubbles (imperceptible to the eye) easily formed due to BSA solutions ability to foam; ii) chamber cleaning that is not efficient enough leaving BSA residues that remain attached to the chamber walls or iii) reductions in the BSA concentration, since BSA adsorbs easily to the chamber walls and tubing.

As a first approach to predict the behavior of k and α for a solution, the data is fitted to Jordan empiric expression. The Jordan expression fits adequately both the thermal conductivity and diffusivity experimental data. From that, thermal conductivity and thermal diffusivity of (pure) BSA is estimated to be 0.20 W/Km and $0.64 \times 10^{-7} \text{ m}^2/\text{s}$, respectively.

6. Conclusion

In this paper, we demonstrate a fluidic chip-calorimeter for simultaneous measurement of thermal conductivity and diffusivity of 2.5 μl bio-chemical fluids using ac calorimetry.

A calibration of the micro-calorimeter was performed to relate the output voltage of the thermopile with the calibration liquid thermal properties. A simplified model for the heat transfer in the calorimeter-liquid system was developed. The model indicates that most of the heat waves propagate through the liquid on the direction perpendicular to the membranes. The chip calorimeter shows 5 V/W sensitivity and 200 K/W thermal resistance for water.

Thermal properties of methanol-water mixtures were measured for methanol volume concentrations between 0.1 % and 1 %. Thermal conductivity and diffusivity are reproducibly measured within ~ 0.3 % error. Experimental data behavior follows Jordan empiric equation correlating the mixture thermal properties with the volume fraction of water and methanol. The micro-calorimeter resolves thermal conductivity and thermal diffusivity down to 1 % methanol concentrations. Such high resolution for methanol-water system was only achieved before using a different technique where the required sample volume is about one order of magnitude higher.

Thermal properties of aqueous BSA solutions can be determined and discriminated for BSA concentration higher than 1% and the intrinsic measurement error is approximately 1.5%. Values for thermal conductivity and diffusivity of (pure) BSA were estimated to be 0.20 W/Km and $0.64 \times 10^{-7} \text{ m}^2/\text{s}$.

The chip calorimeter introduced here shows high potential for lab-on-a-chip applications by enabling thermal characterization of μl liquid samples with high sensitivity and easy sample manipulation.

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